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14. ABSTRACT

Human T-lymphotropic virus type I (HTLV-I) infection is associated with infective dermatitis of childhood. Early childhood infection is also thought to play a role in development of a rare malignancy associated with HTLV-I, adult T-cell leukemia/lymphoma (ATL). ATL develops in <5.0% of persons infected with HTLV-I in childhood. Identifying markers associated with increased risk of ATL among infected persons could be used to target persons for early clinical intervention. Several case reports have documented ATL patients with childhood histories of infective dermatitis. Infective dermatitis may be a cutaneous marker of risk for ATL. There may be additional health effects of HTLV-I infection in children that could be potential markers of risk for development of ATL, however this age group has not been well studied. A cohort study of 28 HTLV-I infected and 280 uninfected children born to women who attended one of two antenatal clinics in Kingston, Jamaica between January 1989 and August 1990 were enrolled in this study. Children received physical examinations and phlebotomy at clinic visits scheduled from six weeks to ten years of age. The primary analysis compared incidence rates for targeted health outcomes between HTLV-I infected and uninfected children. Based on the results of the primary analysis, the secondary analysis examined associations of HTLV-I-associated health outcomes with pre-diagnostic levels of viral and immunologic markers, as well as host genetic markers among HTLV-I infected children. Additionally, levels of immunologic markers at the time of diagnosis were described in infected children with a specific health outcome. HTLV-I infected children had significantly increased incidence rates of seborrheic dermatitis, eczema and hyperreflexia of the lower limbs compared to HTLV-I uninfected children. Additionally, compared to uninfected children, HTLV-I infected children had elevated rates of lymphadenopathy, severe anemia, abnormal lymphocytes and a decreased rate of eosinophilia that were of borderline statistical. Among infected children, seborrheic dermatitis and severe anemia were associated with elevated HTLV-I proviral loads in pre-diagnostic specimens. Children with seborrheic dermatitis had elevated pro-inflammatory cytokines at the time of diagnosis, but not 12 months postinfection. Health outcomes among infected children were not associated with the HLA Class II alleles studied. HTLV-I infection in children may be associated with seborrheic dermatitis and eczema. Further study is needed to confirm these associations and clinically define these HTLV-I associated diseases. Other abnormalities associated with HTLV-I have previously been reported in association with HTLV-I in adults. HTLV-I infected children with seborrheic dermatitis, eczema or severe anemia had elevated levels of proviral load 12 months post-infection that were of borderline statistical significance. At the time of diagnosis, infected children with seborrheic dermatitis had elevated levels of pro-inflammatory cytokines. HTLV-I associated seborrheic dermatitis may be an obvious marker of immune system dysregulation in children.

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Abstract

A Cohort Study of Health Effects of HTLV-I Infection in Jamaican Children and their Associations with Viral, Immunologic and Host Genetic Markers

Elizabeth M. Maloney, Dr. P.H., 2002

Dissertation directed by Terry L. Thomas, Ph.D., National Cancer Institute; Gerald Quinnan, M.D., Leonelo Bautista, Dr. P.H. and Paul Hshieh, Ph.D., Department of Preventive Medicine and Biometrics, USUHS; Chou-Zen Giam, Ph.D., Department of Microbiology and Immunology, USUHS; Lois LaGrenade, M.D., M.PH., Food and Drug Administration

Statement of the problem: Human T-lymphotropic virus type I (HTLV-I) infection is associated with infective dermatitis of childhood. Early childhood infection is also thought to play a role in development of a rare malignancy associated with HTLV-I, adult T-cell leukemia/lymphoma (ATL). ATL develops in <5.0% of persons infected with HTLV-I in childhood. Identifying markers associated with increased risk of ATL among infected persons could be used to target persons for early clinical intervention. Several case reports have documented ATL patients with childhood histories of infective dermatitis. Infective dermatitis may be a cutaneous marker of risk for ATL. There may be additional health effects of HTLV-I infection in children that could be potential markers of risk for development of ATL, however this age group has not been well studied.

Methods: A cohort study of 28 HTLV-I infected and 280 uninfected children born to women who attended one of two antenatal clinics in Kingston, Jamaica between January, 1989 and August, 1990 were enrolled in this study. Children received physical examinations and phlebotomy at clinic visits scheduled from six weeks to ten years of

age. The primary analysis compared incidence rates for targeted health outcomes between HTLV-I infected and uninfected children. Based on the results of the primary analysis, the secondary analysis examined associations of HTLV-I-associated health outcomes with pre-diagnostic levels of viral and immunologic markers, as well as host genetic markers among HTLV-I infected children. Additionally, levels of immunologic markers at the time of diagnosis were described in infected children with a specific health outcome.

Results: HTLV-I infected children had significantly increased incidence rates of seborrheic dermatitis, eczema and hyperreflexia of the lower limbs compared to HTLV-I uninfected children. Additionally, compared to uninfected children, HTLV-I infected children had elevated rates of lymphadenopathy, severe anemia, abnormal lymphocytes and a decreased rate of eosinophilia that were of borderline statistical. Among infected children, seborrheic dermatitis and severe anemia were associated with elevated HTLV-I proviral loads in pre-diagnostic specimens. Children with seborrheic dermatitis had elevated pro-inflammatory cytokines at the time of diagnosis, but not 12 months post-infection. Health outcomes among infected children were not associated with the HLA Class II alleles studied.

Conclusions: HTLV-I infection in children may be associated with seborrheic dermatitis and eczema. Further study is needed to confirm these associations and clinically define these HTLV-I associated diseases. Other abnormalities associated with HTLV-I have previously been reported in association with HTLV-I in adults. HTLV-I infected children with seborrheic dermatitis, eczema or severe anemia had elevated levels of

proviral load 12 months post-infection that were of borderline statistical significance. At the time of diagnosis, infected children with seborrheic dermatitis had elevated levels of pro-inflammatory cytokines. HTLV-I associated seborrheic dermatitis may be an obvious marker of immune system dysregulation in children.

A COHORT STUDY OF HEALTH EFFECTS OF HTLV-I INFECTION IN JAMAICAN CHILDREN AND THEIR ASSOCIATIONS WITH VIRAL, IMMUNOLOGIC AND HOST GENETIC MARKERS

by

Elizabeth Margaret Maloney

Dissertation submitted to the Faculty of the Department of Preventive Medicine and Biometrics Graduate Program of the Uniformed Services University of the Health Sciences in partial fulfillment of the requirements for the degree of

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DEDICATION

To my parents, James and Elinor Maloney, sisters Irene, Pat, Jane, Sara, Catherine (thanks for the laptop) and brothers Jim, Dan and Terry who have always been proud of my accomplishments.

Table of Contents

Approval Sheet i
Copyright Statement ii
Abstract iii
Title Page vi
Acknowledgements vii
Dedication ix
HTLV-I associated diseases and their associations with viral, immunologic and host genetic markers
Introduction and Review of the Literature 1
Introduction. 2
Role of the Candidate. 3
Review of the Literature. 3
The Epidemiology of HTLV-I. 4
HTLV-I associated Disease: ATL. 5
HTLV-I associated Disease: HAM/TSP. 6
HTLV-I associated Disease: Uveitis. 7
Other HTLV-I associated Diseases. 7
HTLV-I Infection in Children. 8
HTLV-I associated Disease in Childhood. 10
HTLV-I Viral Markers. 13
Markers of Immune System Activation. 15
Summary. 16
References. 19

First Manuscript
A Cohort Study of Health Effects of HTLV-I Infection in Jamaican Children. 34
Abstract. 35
Introduction. 36
Materials and Methods. 38
Study Population. 38
Data Collection. 38
Laboratory. 40
Statistical Methods. 42
Results. 43
Interim Medical History. 43
Physical Examination. 44
Hematologic Factors. 47
Discussion. 50
References. 56

Second Manuscript
HTLV-I Proviral Load is associated with Development of Seborrheic Dermatitis in Jamaican Children. 74

Abstract. 75
Introduction. 76
Materials and Methods. 77
Study Population. 77
Data Collection. 78
Laboratory. 79
Viral Markers. 81
Immunologic Markers. 81
Host Genetic Markers. 81
Statistical Methods. 82
Results. 83
Discussion. 86
References. 91

Overall Discussion. 100
Public Health Implications. 111
References. 116

List of Tables

First Manuscript

Table 1: Distribution of Demographic Factors among HTLV-I Infected and HTLV-I Uninfected Jamaican Children. 62

Table 2: Distribution of Health Outcomes among HTLV-I Infected and Uninfected Jamaican Children, and associated Rate Ratios. 63

Table 3: Mean Ages of Diagnosis with HTLV-I Associated Health Outcomes, by HTLV-I Status 65

Second Manuscript

Table 1: HTLV-I Viral Markers and Cytokine Levels in 28 HTLV-I Infected Children from Jamaica. 96

Table 2: Distribution of Viral Markers by Health Outcomes and their Associated Risk Ratios and Confidence Intervals. 97

Table 3: Distribution of HLA Class II Alleles by Health Outcomes. 98

List of Figures

First Manuscript

Figure 1: Venn Diagram of Overlapping Health Outcomes among HTLV-I Infected Children 66

Second Manuscript

Figure 1: Correlation of HTLV-I antibody titer and proviral load. 99

Appendix

Appendix A: Survival Curves for Eczema by HTLV-I Status. 67

Survival Curves for Seborrheic Dermatitis by HTLV-I Status. .68

Survival Curves for Persistent Hyperreflexia by HTLV-I Status. 69

Survival Curve for Lymphadenopathy by HTLV-I Status 70

Survival Curve for Severe Anemia by HTLV-I Status 71

Survival Curve for Eosinophilia by HTLV-I Status 72

Survival Curve for Abnormal Lymphocytes by HTLV-I Status 73

HTLV-I Associated Diseases

and their Associations with Viral, Immunologic and Host Genetic Markers

Elizabeth M. Maloney

Introduction

Human T-cell leukemia/lymphoma virus type I (HTLV-I) is the first retrovirus to be associated with human malignancy and is etiologically linked to adult T-cell leukemia/lymphoma (ATL). HTLV-I is also associated with a neurologic disease called HTLV-I associated myelopathy or tropical spastic paraparesis (HAM/TSP), and an eye disease, uveitis. These diseases are rare with <5% chance of HTLV-I carriers developing ATL or HAM/TSP in their lifetime. Identifying interim markers that predict this fatal (ATL) and debilitating (HAM/TSP) disease is a focus of current epidemiologic studies of HTLV-I. Several case reports suggest that infective dermatitis, a severe HTLV-I-associated dermatitis of childhood, may be a cutaneous marker for later development of ATL. There may be other HTLV-I associated diseases of childhood, however this age group has not been well studied. In a study that compared HTLV-I prevalence in children with infective dermatitis to children with atopic eczema, 14% of the children with atopic eczema were seropositive for HTLV-I, which is much higher than the expected prevalence of <1.7 % in this age group. These data suggest that there may be other disease manifestations of HTLV-I in childhood. A cohort of children born to HTLV-I seropositive and seronegative Jamaican women was followed prospectively for ten years in order to assess disease development and its association with viral, immunologic and host genetics markers.

This study evaluates risks of targeted health outcomes associated with HTLV-I infection by comparing incidence density rates of health outcomes in infected and uninfected children prospectively followed since birth. Based on the results of this primary analysis, HTLV-I infected children are studied to assess whether diseases and

conditions suggested to be associated with HTLV-I are associated with HTLV-I viral and immunologic marker levels obtained 12 months post-infection. Additionally, this study examines the association of candidate human leukocyte antigen (HLA) Class II alleles with HTLV-I-associated health outcomes and conditions suggested by our primary analysis.

Role of the Candidate

This cohort study had achieved ten years of prospective clinical observation and specimen collection at the time the candidate made her contribution. The candidate conducted an independent review of the literature as a basis for independently designing the hypotheses examined in these two analyses. Additionally, the candidate independently wrote the review of the literature. The candidate contributed to data collection by abstracting laboratory data on-site in Jamaica in order to clarify the type of anemia associated with HTLV-I. The candidate independently negotiated with collaborators to facilitate the laboratory testing of HTLV-I proviral load, antibody titer, serum cytokines and HLA Class II alleles. Additionally, the candidate independently performed all statistical analyses, interpreted the data, synthesized its significance, wrote the manuscripts and developed future research plans based on the results of this study.

Review of the Literature

HTLV-I

Human T-cell lymphotropic virus type-I (HTLV-I) is the first retrovirus to be associated with malignancy in man. It was first discovered in 1980 when detected in cell lines obtained from a patient initially diagnosed with cutaneous T-cell lymphoma and

subsequently determined to have mycosis fungoides [1], and a patient with Sezary syndrome, also a T-cell malignancy [2]. In retrospect, these patients were determined to be misdiagnosed and were reclassified as adult T-cell leukemia/lymphoma (ATL), first described in 1977 in Japan [3]. In surveys of ATL patients from the West Indies and southwestern Japanese islands of Kyushu and Shikoku, almost all (90-100%) had antibody reactivity to HTLV-I antigen [4,5].

HTLV-I is an enveloped retrovirus with type-C morphology, characterized by particle morphology and the pattern of 'budding' virus particles (virions) from the surface of HTLV-I infected cells [1]. HTLV-I has an RNA genome, which is reversed-transcribed into DNA by reverse transcriptase, and inserted into the host cellular genome as HTLV-I provirus. Once integrated, HTLV-I genome remains for the life of the target cell and host. The HTLV-I genome consists of three structural genes (*gag, pol, env*), *pro*, four open reading frames (orfs) and long terminal repeat regions (LTR) [6]. *Gag* codes for the major structural proteins of the virion core and *pro* encodes the viral protease. Viral protease cleaves the Gag precursors into mature proteins: the matrix protein (MA) p19, the capsid protein (CA) p24, and the nucleocapsid protein (NC) p15 [7]. *Pol* codes for DNA polymerase (reverse transcripase) and *env* codes for envelope glycoproteins (gp21 and gp46). X-1 orf codes for p12, x-II orf codes for p30Tof and p13, x-III orf codes for $p27^{rex}$ and $p21^{rex}$ and x-IV orf codes for $p40^{tax}$. The regulatory protein, $p40^{tax}$ regulates viral protein expression and interacts with host cellular genes controlling cell division, thus controlling viral replication via these mechanisms. HTLV-I is highly cell-associated and T-cell tropic, preferentially infecting CD4+ T-cells [6].

The Epidemiology of HTLV-I

Geographically, HTLV-I is endemic in parts of the Caribbean, southern islands of Japan, parts of Africa and South America and the Pacific islands of Melanesia and Papua New Guinea. Much of the epidemiology of this virus was established by population-based studies conducted in the Caribbean islands of Jamaica and the southwestern Japan community of Miyazaki, where prevalence rates are 6% and 30%, respectively [8-10]. Prevalence increases with age and is higher in adult females compared to adult males. In Jamaica, the prevalence rises from 1.7% (10-19 years of age) to 9.1% ($\geq$ 70 years) in men, and from 1.9% (10-19 years) to 17.4% ($\geq$ 70 years) in women, with female seroprevalence exceeding males starting in the second decade [9]. In Japan, the increased seroprevalence in females compared to males occurs after the fifth decade [10]. HTLV-I seropositivity is associated with markers of low socioeconomic (SES) status [9].

Modes of HTLV-I transmission routes are similar to those for HIV and include maternal-child, transfusion and sexual routes. HTLV-I is transmitted from infected women to their offspring predominantly via breast milk, with seroconversion occurring in 18%-26% of the breastfed offspring [11-15]. HTLV-I is also transmitted via transfusion of infected blood products. Risk factors for transfusion transmission in Jamaica include short shelf-life of blood unit (< one week); current use of immunosuppressive medications and male gender [16]. Approximately 44% of recipients of HTLV-I seropositive cellular units seroconvert within two months of transfusion [16]. Sexual transmission of HTLV-I is another predominant route of infection, with male-to-female transmission considered to be four times more likely than the reverse [17,18]. The rate of

transmission of HTLV-I via the sexual route between couples is reported to be 60% over 10 years of exposure [18].

HTLV-I Associated Disease: ATL

HTLV-I is etiologically associated with adult T-cell leukemia/lymphoma (ATL), a rare T-cell malignancy characterized by hypercalcemia, hepatomegaly, splenomegaly, lymphadenopathy, skin involvement and presence of abnormal lymphocytes. ATL patients have evidence of a monoclonal integration of HTLV-I proviral DNA within leukemia cells and a proliferation of CD4+CD25+ cells [19]. There are four subtypes: acute, lymphomatous, chronic and smoldering. The first two subtypes are associated with a rapidly progressive course with a median survival time of approximately 20 weeks. Smoldering and chronic ATL have a more indolent course [20]. In Jamaica, the mean age at diagnosis of ATL is 43 years of age [20] and the incidence rate is estimated as 2 per 10^5 general population and 16 per 10^5 HTLV-I carriers per year and is higher (75 per 10^5 carriers per year) among individuals infected in childhood [21,22]. This fact, in addition to the finding that all mothers of ATL patients are themselves HTLV-I seropositive, support a role for early childhood infection as a risk factor for development of ATL in adulthood [23]. Additionally, ATL has been associated with a specific human leukocyte antigen (HLA) haplotype in both Japan and Jamaica, DRB1*1501-DQB1*0602, arguing for a common association of this haplotype across races [24,25].

HTLV-I Associated Disease: HAM/TSP

HTLV-I was associated with a chronic neurologic disease called HTLV-I associated myelopathy/tropical spastic paraparesis (HAM/TSP) in 1985 [26]. HAM/TSP is characterized by weakness and hyperreflexia of the lower limbs, bladder dysfunction and

impotence. The mean age at onset of disease is 47 years in Jamaica [27] and the incidence is 3 per 10^5 general population, or 22 per 10^5 HTLV-I carriers per year [28]. Although HAM/TSP is not fatal, it is progressively debilitating. Risk factors for HAM/TSP include sexual factors (number of lifetime partners; early age of initiating sex) and blood transfusion with infected units, suggesting that adult acquisition of HTLV-I infection plays an important role in disease development [27,29,30]. Host genetics are also suggested to play a role, as HLA Class II alleles DRB1*0101 (DR1) and DQB1*0501 (DQ1) were detected more frequently among HAM/TSP patients compared to asymptomatic carriers [24].

HTLV-I Associated Disease: Uveitis

Uveitis is an inflammatory disease in the eye that responds to corticosteroid therapy but relapses post-treatment at intervals of two months to two years [31]. It has long been associated with infectious and non-infectious diseases, however a significant proportion of the 40% of uveitis patients previously considered idiopathic were associated with HTLV-I [30,32]. The mean age of patients with HTLV-I-associated uveitis (HAD) is 43, although risk of uveitis associated with HTLV-I was significantly greater among younger patients (20-49 years) [32]. The incidence of uveitis in Japan is similar to that of ATL, however the incidence of uveitis in Jamaica is unknown.

Other HTLV-I Associated Diseases

It has also been suggested that HTLV-I is associated with Sjögren's syndrome, arthropathy, polymyositis and lymphadenitis [33-37]. However, more subtle diseases have been associated with HTLV-I seropositivity in studies of carriers. HTLV-I has been

significantly associated with detection of mild anemia among elderly Japanese men who immigrated to Hawaii, while a statistically non-significant increased risk of mild anemia was detected among males participating in the Miyazaki, Japan cohort study [38,39]. In Jamaica HTLV-I was associated with severe anemia, although statistical significance was not achieved [40]. A significantly decreased eosinophil percent was associated with HTLV-I in Jamaica and Japan [41,42]. Lymphopenia was associated with HTLV-I in elderly male Japanese emigrants to Hawaii, while lymphoctyosis was associated with HTLV-I among Japanese adults in Miyaziki [38,42]. Other HTLV-I associated conditions reported among the Miyazaki cohort include asthma among males and abnormal cardiac electrocardiogram (ECG), which supports an earlier report of history of cardiac disease in association with HTLV-I [39,10]. HTLV-I seropositivity was associated with lymphadenopathy among a cohort of U.S. blood donors [43]. In this same cohort, HTLV-I was associated with an increased incidence of bladder or kidney infections, which supports the association of HTLV-I with history of kidney disease in the Miyazaki, Japan cohort [44,39].

With the exception of uveitis, all HTLV-I associated diseases have skin conditions preceding or concurring with disease onset. ATL often presents as a skin disease (40%-45% of cases) that includes generalized papulonodular rash and placques, erythroderma, vesicularbulbous lesions, psoriasis, erythroderma and ichthyosiform rash [45]. Several case reports of ATL patients document a history of skin conditions that precede ATL diagnosis by as many as 21 years [46-48]. Among five ATL patients who presented with skin lesions, the histology of the skin lesions was variable and consisted of histiocytes, plasma cells, eosinophils, atypical mononuclear cells and lymphocytes with evidence of

epidermotropism but HTLV-I proviral integration was present in skin biopsies [49]. A history of skin conditions is also documented in case reports of HAM/TSP patients [50-53].

HTLV-I Infection in Children

The seroprevalence of HTLV-I infection in children ≤ ten years of age in endemic areas is approximately 1% [54]. HTLV-I infection in this age cohort is due to maternal-child transmission, as prevalence among children of HTLV-I seropositive mothers remains steady over two-year age strata in children ≤ 13 years of age [55], supporting transmission within the first two years of life. This age of infection is also supported by data obtained from prospective studies of maternal-child transmission which reveal infection to occur at a mean of 14 months of age (range: 4 – 27 months) in Jamaica, and similarly between six months to two years in Kagoshima, Japan [11,56]. Maternal antibody (HTLV-I IgG, but not IgM) is evident in virtually all cord blood specimens from children born to HTLV-I seropositive mothers, but wanes between 6 – 18 months [13,55,11,56]. While higher maternal antibody level has been associated with decreased maternal-child transmission of HTLV-I in bottle-fed babies, breastfeeding duration beyond the waning of maternal antibody is believed to result in maternal child transmission [57,11,55]. In Jamaica, the maternal-child transmission rate in children of seropositive mothers who breastfed for < 12 months was 9%, compared to a rate of 32% among children who breastfed for ≥ 12 months [11]. This effect of breast feeding duration is supported in all studies that examined this association, and appears to be the strongest risk factor for maternal-child transmission [12-15,55,56,13]. However,

seroconversion occurs in 3-6% of bottle-fed children, indicating that breast milk is not the exclusive vehicle of maternal-child transmission [55,56].

Additional independent risk factors associated with maternal-child transmission in Jamaica include low maternal income level, longer duration of ruptured membranes during delivery, high maternal proviral load and maternal antibody titer [11,57]. Similarly, both maternal proviral load and maternal antibody titer were significantly associated with maternal-child transmission in French Guiana [58]. Maternal anti-tax antibody, which is associated with sexual transmission between spouse pairs, has also been associated with maternal-child transmission [59-61]. Low maternal income may be a marker of sanitation or nutrition status, or could be associated with other infections and consequent antigenic stimulation. Duration of ruptured membranes (>four hours) as a risk factor for transmission suggests that risk is associated with exposure to maternal blood at the time of delivery. However, this was not supported by an examination of seroconversion times in children born to mothers with a short or long duration of ruptured membranes [11]. Thus it is uncertain how this risk factor should be interpreted.

In French Guiana, a family study of HTLV-I transmission in two villages used segregation analysis to support a role for a dominant gene predisposing to HTLV-I infection, which the authors claimed explained most of the HTLV-I infection in children younger than ten years of age [62]. The authors of this study are now conducting studies to identify the dominant gene.

While clinical latency in adults is measured in decades, latency in children could be much shorter. If another retrovirus, HIV can be used as an example, HIV infected adults experience eight to ten years latency, while children who are vertically infected with HIV

may develop signs and symptoms of disease in their first year [63]. Thus the prospective study of HTLV-I infected children may inform us of the pathogenesis and natural history of disease in HTLV-I infected adults.

HTLV-I Associated Disease in Childhood

Infective dermatitis in children was first linked with HTLV-I infection in Jamaica in 1990, although it was reported as a unique dermatitis as early as 1966 and 1967 [64-66]. Diagnostic criteria for infective dermatitis were described in 1998 and include: eczema of the scalp, axillae and groin, retroauricular areas, eyelid margins, paranasal area and/or neck; chronic watery nasal discharge without other signs of rhinitis and/or crusting of anterior nares; relapsing recurrence upon withdrawal of antibiotic treatment; onset in childhood and HTLV-I seropositivity [67]. Patients with infective dermatitis usually have concurrent infections with staphylococcus aureus and/or B-hemolytic streptococci of skin or anterior nares, generalized lymphadenopathy, anemia, hyperimmunoglobulinemia and elevated CD4 cell count and erythrocyte sedimentation rate [67]. The age at diagnosis has been reported to range from three to sixteen years [68], however onset of symptoms typically occurs at two years of age (LaGrenade L, personal communication).

HTLV-I infection is the only known risk factor for this form of infective dermatitis although immune dysregulation and host genetic factors are suggested to play a role [67,68]. A Jamaican woman with HAM/TSP and a childhood history of infective dermatitis, shared an HLA Class II haplotype (DRB1*DQB1*(1101-0301) with her son who was diagnosed with infective dermatitis at three years of age [52]. These data are consistent with the association of this haploytpe with HAM/TSP among Japanese

patients, and its association with a 'high immune response' and high antibody titer [69]. HTLV-I associated infective dermatitis has been described in children from Trinidad [70], and individual cases have been reported from Colombia, Dominican Republic, Barbados and Japan [71-74]. In a report that described the diagnostic criteria for infective dermatitis by comparing children with this disease to children with atopic eczema, 14% of the atopic eczema patients were HTLV-I seropositive [67]. This prevalence rate is high compared to the expected prevalence of < 1.7% in this age group and suggests that HTLV-I may be associated with atopic eczema, or skin diseases that range from mild to severe.

Although ATL and HAM/TSP develop predominantly in adults, there have been a series of case-reports of these diseases occurring in children. Six separate publications identified HTLV-I-associated ATL in children who ranged in age from one year to 17 years of age [75-80]. However skin disease was noted at presentation for four of the five children with available clinical information, and three of those four had a history of previous skin disease [75-80]. An additional ten cases of HTLV-I associated ATL were described in Brazilian children ages two years to 18 years. They were clinically similar to adult ATL cases, all having lymphadenopathy, hepatosplenomegaly and marked skin lesions, while four had hypercalcemia. Nine of the ten children had a CD4+/CD25+ phenotype, the typical phenotype of leukemic cells in patients with ATL. Vertical HTLV-I transmission was documented in eight of the ten children. Four cases had deletion of the p16 gene and one had an alteration of the p53 gene, leading the authors to speculate that such deletions /mutations of cellular genes which control proliferation could result in such a short latency period [81].

HAM/TSP has been reported in five Brazilian children ages 11 through 17, who presented to the Institute of Pediatrics of the Federal University of Rio de Janeiro between 1989 and 2000. All but one of these children were breast fed by HTLV-I positive mothers and had a history of dermatitis at time of diagnosis [82]. It is possible that increased surveillance of HTLV-I infected children with skin diseases will result in increased numbers of children with HTLV-I associated diseases.

HTLV-I Viral Markers

HTLV-I proviral DNA level is higher in ATL and HAM/TSP patients compared to HTLV-I seropositive asymptomatic carriers, as measured by copies of HTLV-I proviral DNA per peripheral blood mononuclear cells (PBMC) [83,84], in situ PCR [85], reverse transcriptase (RT)-PCR [86], inverse PCR [87], percent integration of proviral DNA using Southern blot [88] and nested PCR [89]. Proviral DNA load in HAM/TSP patients is as much as 16-fold higher than in carriers [83,84]. These data suggest that proviral load plays an important role in disease development. However, the duration of time during which elevated proviral load precedes disease development has been studied only rarely. ATL patients are usually diagnosed as incident cases, thus their proviral load level is usually measured at onset of clinical disease. However, in one study of three ATL patients who were prospectively studied for six years preceding ATL diagnosis, the median pre-diagnostic proviral load of 4.9 copies per 100 PBMC six years preceding development of ATL was significantly higher than the median proviral load of 0.8 copies per 100 PBMC in age- and sex- matched carrier controls [90,91]. Similarly, most HAM/TSP patients present three to four years after onset of symptoms [28], as disease

progression is generally slow, thus their proviral load levels are usually measured after disease onset. However, in one prospective study of 20 HTLV-I seropositive carriers at high risk of disease, elevation of HTLV-I proviral load preceded development of HTLV-I-associated inflammatory disease by an average of ten months in five carriers [92].

It is unknown whether HTLV-I proviral load is significantly higher in patients with infective dermatitis, although these patients do have significantly elevated antibody titer and a high prevalence of tax-specific antibody, a profile similar to HAM/TSP patients [LaGrenade L et al, personal communication]. By analogy, infective dermatitis patients are expected to have similarly elevated proviral DNA load. The single child who developed infective dermatitis in a Jamaican pediatric cohort had an HTLV-I proviral DNA level at time of infection in the range of that of adult ATL and HAM/TSP patients, suggesting that proviral load may be an important factor in the development of infective dermatitis in children [93].

HTLV-I antibody titer levels are significantly higher in HAM/TSP patients compared to asymptomatic HTLV-I carriers [83], even when examined within strata of HTLV-I proviral load [94]. ATL patients' median antibody titer levels are also significantly higher than those of carriers, but not different from patients with HAM/TSP [83]. This similarity in humoral response to HTLV-I infection by these two disease states is not seen in their cellular immune response to infection. HAM/TSP patients are characterized as having a hyperimmune response to infection and exhibit high levels of spontaneous lymphocytic proliferation (SLP) [95], while ATL patients are characterized as immune suppressed and exhibit SLP levels lower than that detected among carriers [96]. In HAM/TSP patients this hyperimmune response has been further demonstrated

by the high level of tax-specific CD8+ T cells (cytotoxic T cells), which correlated with proviral load [97-100].

The natural history of viral markers has been studied in two adult cohorts of HTLV-I carriers [83,101]. In a prospective study of transfusion recipients of HTLV-I positive blood with known dates of infection, the level of antibody titer established early in infection was linked to the level of proviral DNA load at time of infection and remained at that level [83]. This same steady state of both proviral load and antibody titer charaterized carriers from Miyazaki, Japan who were followed for 11 years after infection [101]. In a cross-sectional study, HTLV-I antibody titer and HTLV-I proviral load were significantly correlated among adult carriers ($r = 0.58, p = 0.04$) [102]. In the single patient with infective dermatitis who arose in this Jamaican cohort of children, his antibody titer level paralleled HTLV-I proviral load over the course of follow-up [93].

The $p40^{tax}$ protein of HTLV-I is believed to play a major role in the pathogenesis of this virus. *Tax* interacts with viral transcriptional enhancer in the *LTR* region of HTLV-I to stimulate viral production. *Tax* also interferes with functions of tumor suppressor genes p53 and p21, perturbs cell cycle progression, impairs DNA-repair mechanisms and causes aberrant proliferation of infected cells [6].

Tax antibody is detected in almost all (94%) of HAM/TSP patients, approximately 60% of ATL patients and 48% of asymptomatic carriers [103,104]. Thus it may be a useful marker in distinguishing carriers who are at risk of developing HAM/TSP. In an animal model, the presence of anti-*tax* antibody was significantly associated with cocultivatable virus in rabbits, an indicator of viral replication [105]. In asymptomatic human carriers, anti-*tax* antibody and HTLV-I proviral DNA load were highly correlated

(r = 0.68) [107]. This association with proviral load likely underlies the significant association between anti-*tax* antibody and sexual transmission [59], as well as maternal-child transmission of HTLV-I [104,60]. None of these viral markers has been studied prospectively in relation to disease development in pediatric carriers.

Markers of Immune System Activation

Cytokines are produced predominantly, but not exclusively, by immune system cells for multiple purposes. They induce T-cell and B-cell growth, activate macrophages, lymphocytes and cytotoxic T-cells, and induce the major histocompatibility complex (MHC) [107]. T helper type 1 (TH-1) cytokines are referred to as pro-inflammatory cytokines and include interleukin-1 (IL-1), interleukin-2 (IL-2), interferon-gamma (IFNγ), tumor necrosis factor-alfa (TNF-α) and others. They function to promote cell-mediated immunity against intracellular pathogens by increasing T-cell proliferation, activating macrophages and increasing lymphocyte/endothelial cell adhesion. T helper type 2 (TH-2) cytokines include interleukin-4 (IL-4), interleukin-5 (IL-5) and interleukin-10 (IL-10), among others, and are associated with T-cell activation of B-cell growth and humoral, anti-parasitic and allergic immune response [108]. Although TH-1 and TH-2 cytokine production are not mutually exclusive, there is usually a predominance of one pathway over another whereby the cytokines produced in a cell-mediated response down-regulate the production of cytokines by a humoral immune response and vice versa [109].

ATL is associated with a TH-2 cytokine response pattern and HAM/TSP is characterized by a TH-1 cytokine response pattern [110]. It is unknown if infective dermatitis is associated with a TH-1 or TH-2 pattern. In vitro studies based on co-cultivation of keratinocytes from seborrheic dermatitis patients with the *Malassezia* yeast,

which causes this skin disease resulted in production of TH-1 cytokines [111]. Because infective dermatitis is a severe form of seborrheic dermatitis, by analogy, we would expect infective dermatitis to be associated with TH-1 cytokines.

Summary

HTLV-I infection in childhood is believed to be an important determinant in development of ATL in adulthood [22]. Since development of ATL occurs in only 1-4% of HTLV-I carriers infected in childhood, it would be useful to identify a marker among carriers that predicts who will develop ATL. Development of HTLV-I-associated disease in childhood may be that marker. Reports of five adults with either ATL or HAM/TSP include record reviews that document a childhood diagnosis of infective dermatitis [53,48]. Thus, HTLV-I associated disease in childhood may be a harbinger for more severe HTLV-I associated disease outcomes in adulthood. Identifying childhood diseases associated with HTLV-I, as well as risk factors for these diseases could contribute to a better understanding of the pathogenesis of HTLV-I by shortening the latency period from decades (in adults) to years (in children).

Our primary objective is the study of health outcomes associated with HTLV-I infection in children. This study is based on the prospective clinical observation of a cohort of 28 HTLV-I infected and 280 uninfected children. These children were born to 305 mothers who enrolled in a study of maternal-child transmission of HTLV-I between January, 1989 and August, 1990 at two antenatal clinics in Kingston, Jamaica. Children were provided a physical examination and phlebotomy at clinic visits scheduled every six weeks for the first three months of life, every three months up to age two years and every six months thereafter until age ten years. Physical examination included a general

examination at each clinic visit, and a skin assessment and a neurologic screening examination beginning at 30 months of age. Phlebotomy products included serum or plasma and lymphocytes for HTLV-I serology and quantitation of HTLV-I proviral load, as well as the collection of blood for computing a complete blood count (CBC) and differential. Targeted health outcomes include eczema and seborrheic dermatitis, because they are the differential diagnostic categories for infective dermatitis. Additional outcomes of interest include diseases and conditions which have been associated with HTLV-I in adults: lymphadenopathy, leukocytosis, lymphocytosis, and abnormal lymphocytes; gait abnormality and hyperreflexia and increased muscle tone of the lower limbs; eosinophilia (which is inversely associated with HTLV-I), anemia, asthma, bacterial infections and cardiac abnormality. Additionally, to investigate the clinical observation that HTLV-I infection is associated with small size for age, we will examine body mass index as an indicator of growth.

Health outcomes suggested to be associated with HTLV-I infection as a result of our primary analysis will be the targeted health outcomes in our secondary objective. Our secondary objective is to examine whether risks for these specific health outcomes are associated with viral, immunologic and host genetic markers among HTLV-I infected children. For this purpose, HTLV-I proviral load, antibody titer and tax-specific antibody were measured in specimens obtained approximately 12 months post-infection in the 28 infected children. Immunologic markers were measured in sera obtained at the same time point and included cytokines that represented TH-1 immune activation and TH-2 immune activation. Host genetic markers included HLA Class II alleles previously associated with ATL or HAM/TSP: DRB1*1101, DQB1*0301 and DQB1*0602.

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First Manuscript

A Cohort Study of Health Effects of HTLV-I Infection in Jamaican Children

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Abstract

Human T-lymphotropic virus type I (HTLV-I) is known to be associated with a malignancy called adult T-cell leukemia/lymphoma (ATL), a chronic neurologic disease called HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP), and an inflammatory eye disease called uveitis. The average age of onset for these diseases is in adulthood. Among children HTLV-I is associated with infective dermatitis (ID), a severe exudative eczema. However, morbidity associated with HTLV-I infection in childhood has not been well studied. In this cohort study we compared rates of disease development in 28 HTLV-I infected and 280 uninfected children clinically followed from age six weeks to a maximum of ten years. HTLV-I infected children had a significantly increased risk of developing seborrheic dermatitis, eczema and persistent hyperreflexia (RR = 4.2, CI = 1.7-10.2, RR = 2.9, CI = 1.8-4.9, RR = 3.6, CI = 1.6-8.1, respectively). Additionally, HTLV-I infected children had increased risks of developing some of the less severe diseases and conditions linked to HTLV-I infection among adults: severe anemia (RR = 2.5, CI = 0.8-7.9), lymphadenopathy (RR = 1.7, CI = 1.0-2.9) and abnormal lymphocytes (RR = 1.8, CI = 0.7-4.9). Also, as reported for adults, HTLV-I infected children had a decreased risk of developing eosinophilia (RR = 0.6, CI = 0.3-1.2). Our study suggests that HTLV-I infection in childhood may be associated with seborrheic dermatitis, eczema and persistent hyperreflexia. Additionally, these data suggest that some of the conditions associated with HTLV-I in adulthood are also associated with HTLV-I infection in childhood.

Introduction

Human T-cell lymphotropic virus type I (HTLV-I) causes a rare, but almost uniformly fatal T-cell malignancy called adult T-cell leukemia/lymphoma (ATL), and a chronic debilitating neurologic disease called HTLV-I associated myelopathy or tropical spastic parapresis (HAM/TSP) [1,2]. HTLV-I is also associated with an inflammatory eye disease, HTLV-I-associated uveitis, and is suggested to be associated with several other inflammatory diseases including arthropathy, Sjogren's syndrome, lymphadenitis and polymyositis [3-7]. These diseases occur predominantly among adults. Less severe conditions associated with HTLV-I among adults include subtle forms of some of the clinical signs of ATL and HAM/TSP. Lymphadenopathy and lymphocytosis have been associated with HTLV-I seropositivity in adults and are also components of a constellation of signs and symptoms that define ATL [8,9]. Gait abnormality, a clinical feature of HAM/TSP was associated with HTLV-I infection in U.S. blood donors [8,10]. Other conditions associated with HTLV-I carrier status among adults include asthma, cardiac abnormality, bacterial infections, anemia, and decreased prevalence of eosinophilia [8,9,11-14].

Among children, HTLV-I is etiologically associated with infective dermatitis, a severe exudative eczema with onset between ages three and sixteen years, and abatement in puberty [15]. While 100% of a group of children with infective dermatitis in Jamaica were found to be HTLV-I seropositive, 14% of a control group of children with atopic eczema were also HTLV-I positive [16]. This prevalence among children with atopic eczema is higher than the expected prevalence of <1.7% in this age group. HTLV-I may be associated with a broad range of skin diseases in childhood.

We sought to study health effects of HTLV-I in a cohort of children born to HTLV-I seropositive and seronegative women and clinically observed from six weeks of age to a maximum age of 10 years. Specifically, we examined whether, compared to HTLV-I uninfected children, HTLV-I infected children were at a significantly increased risk of developing the signs and symptoms associated with ATL and HAM/TSP, as well as other morbidity associated with HTLV-I in adults. Thus we examined the association of HTLV-I with lymphadenopathy and other signs of ATL, including leukocytosis, lymphocytosis and the presence of abnormal lymphocytes. Additionally, we examined the association of HTLV-I with gait abnormality and other signs of HAM/TSP, including hyperreflexia of the lower limbs, increased muscle tone and decreased muscle power. We also assessed whether HTLV-I infected children were at increased risk of developing anemia, asthma and bacterial infections and decreased risk of developing eosinophilia. Because of the association of HTLV-I with infective dermatitis in children, we examined whether HTLV-I infected children had an increased risk of developing skin diseases that represent differential diagnostic categories for infective dermatitis: eczema and seborrheic dermatitis. Finally, because Jamaican dermatologists have observed that children with infective dermatitis appeared small for their age, we examined the relationship between HTLV-I infection and physical growth by analyzing body mass index (BMI).

Materials and Methods

Study Population

A major objective of this study was to measure health effects associated with HTLV-I infection in childhood. The study population was described in detail by Wiktor SZ, et al. [17]. Briefly, 9,430 pregnant women attending either of two antenatal clinics in Kingston, Jamaica between January, 1989 and August, 1990 were screened for HTLV-I antibodies. Seropositive status was determined for 350 (3.7%) women and 212 were enrolled along with 145 seronegative women. At enrollment, mothers donated a blood sample and were interviewed regarding demographic information, pregnancy and sexual history. Of the 357 children born to enrolled mothers, 308 children achieved at least one clinic visit, including 28 children who became HTLV-I infected, and 280 children who remained seronegative. These children underwent physical examination and phlebotomy, while mothers responded to questions related to their children's interim health at clinic visits that occurred every six weeks for the first six months of life, then every three months up to two years of age, and every six months thereafter up to ten years of age.

Data Collection

Clinical evaluation of the enrolled children included a general physical examination, skin assessment and neurologic screening examination conducted by a pediatrician at scheduled clinic visits. Skin examination for rashes was conducted at clinic visits from six weeks through 24 months. A more comprehensive skin assessment was conducted at clinic visits between 30 months and 120 months of age. A neurologic screening examination was conducted from age 30 months to 120 months to assess overall muscle

tone, power and gait abnormality, as well as upper and lower body reflexes, weakness and pain.

Children's interim health histories were obtained from mothers by a trained nurse using a standardized questionnaire. Mothers were queried concerning rash and ear infection at clinic visits occurring at six weeks of age to 120 months of age. At clinic visits from 30 months to 120 months, mothers were also asked to report if their children had pneumonia or asthma since their last clinic visit. Interval reports of diarrhea were obtained from ages six weeks to ten years. Diarrhea was not expected to be associated with HTLV-I infection and served as a means of assessing the presence of observer bias in this study.

Growth parameters, including height and weight were obtained by a nurse throughout the entire period of clinical observation, however height measurements obtained at age two years and younger were assessed as invalid thus analysis was based on measurements obtained from 30 months to 120 months of age. Body mass index (BMI) was calculated as $(\text{weight}/\text{height})^2$ and abnormally low BMI was defined as less than the 5^{th} percentile for BMI, according to the National Center for Health Statistics. These values for girls included: BMI<14.0 (ages 30 – 36 months); BMI<13.8 (ages 42 - 54 months); BMI<13.6 (ages 60 – 66 months); BMI<13.4 (ages 72 – 90 months); BMI<13.8 (ages 96 – 114 months); BMI<14.0 (ages 120 months). Abnormally low values for boys included: BMI<14.4 (ages 30 – 36 months); BMI<14.2 (age 42 months); BMI<14.0 (ages 48 – 54 months); BMI< 13.8 (ages 60 – 102 months); BMI<14.0 (ages 108 – 114 months); BMI<14.2 (age 120 months).

Information on maternal demographic factors was obtained by trained nurses at the time of enrollment. Total income was dichotomized as high (≥ $100 Jamaican per week) and low (< $100 Jamaican per week), based on the 1989 currency exchange rate of $5.50 Jamaican = $1.00 U.S.

Laboratory

At clinic visits occurring during the first 24 months heparinized blood was collected from the children by heel-stick in capillary tubes. Whole blood was centrifuged to separate serum and lymphocytes. Serum was used for HTLV-I serologic testing; lymphocytes were used for measuring HTLV-I provirus. From age 30 months to 120 months, 10cc of venous heparinized blood was collected and processed as described above for the same purposes. An additional 3.5 cc of blood was collected in an EDTA tube for complete blood count (CBC) and differential. Specimens were stored at –70° C until withdrawal for testing. CBC and differential were measured by trained clinical laboratory technicians using standard methods. Age-specific normal values for Jamaican children were used to determine abnormally high and low blood counts for all CBC parameters. Severe anemia was defined as: hemoglobin (Hgb) < 9.0 g/dl (ages 30 – 36 months); Hgb< 9.6 g/dl (ages 42 – 66 months) and Hgb< 10.0 g/dl (ages 72 – 120 months). White blood cell count (WBC) was examined for high values, defined as WBC > 15.5 x10^3 (ages 30 - 66 months); WBC> 8 x 10^3 (ages 72 - 114 months); WBC> 13.5 x 10^3 (age 120 months). Lymphocyte count was examined for abnormally high values (> 8,500 per μl, ages 30 - 120 months). Eosinophilia was defined as ≥ 800 eosinophil count per cc of blood. The number of abnormal lymphoyctes per 100 cells was determined by microscope review of peripheral blood smear

HTLV-I viral status

HTLV-I antibody testing of mothers and offspring had previously been conducted using ELISA (Cambridge Bioscience, Cambridge, MA; Dupont, Wilmington, DE; or Genetic Systems, Seattle WA) and Western blot (Cambridge Bioscience) [17]. Children who were seropositive by ELISA from 12 to 24 months of age were considered screen positive. Serial samples that preceded the first seropositive samples were tested by Western blot to identify the estimated date of seroconversion. Western blot confirmation of seropositivity required evidence of reactivity against the p24 capsid and envelope proteins.

HTLV-I quantitative proviral DNA testing of serial lymphocyte specimens surrounding the estimated date of seroconversion was used to verify the age of infection, determined to be midpoint between the draw dates for the first sample with a positive PCR test and the last sample with a negative PCR test. DNA was extracted from $1x10^6$ cryopreserved peripheral blood mononuclear cells (PBMC) using the PureGene DNA isolation Kit (Gentra Systems, Inc. Minneapolis, MN). Proviral load was measured in 10 μl samples of solutions containing 300 ng of DNA using an ABI PRISM 7700 Sequence Detection System and Taqman PCR Reagent [P/N N808-0230] (Perkin Elmer Applied Biosystems, Foster City, CA) in a 96-well format. The HTLV-I/II primers were highly conserved sequences from the *tax* gene and are designated as HTV-F5 and HTV-R4 (GenBank Accession Nos. 7358-7378 and 7518-7499). Triplicate reactions were performed and genome copy numbers were calculated by interpolation from the plasmid control regression curve. This assay reliably detects at least 3 copies/10^5 lymphocytes [18]. HTLV-I proviral load was normalized for the number of lymphocytes in each

sample by dividing the number of provirus copies by twice the count of human endogenous retrovirus 3 (HERV-3), which exists as two copies per cell**.** Primer pairs for HERV-3 included PHP10-F and PHP10-R. Children who maintained HTLV-I seronegative status for the first three years of life were not further tested by Western blot or PCR, and are assumed to be uninfected.

Statistical Methods

Among HTLV-I infected children, person-years (PY) at risk for health outcomes accumulated from their estimated date of infection to their date of diagnosis with a specific health outcome or their date of last clinic visit, the end of the study period or their date of death. Among HTLV-I seronegative children, PY at risk for health outcomes accrued from the date of their first clinic visit to the same endpoints as described for infected children. However, because measurement of hematologic and neurologic factors was initiated at 30 months, PYs at risk for these outcomes accrued from the 30 month clinic visit for both uninfected and infected children, as the oldest age at infection among infected children was 27 months. Cox proportional hazards analysis was used to determine the incidence density rates of health outcomes among HTLV-I infected children compared to HTLV-I uninfected children. Separate models were developed for each health outcome, which was regressed on HTLV-I infection status. Examination of potential confounding by child's sex and maternal income was examined by including child's sex and dichotomous levels of maternal income in the regression models. Rate ratio (RR) was used to measure the effect of HTLV-I infection on risk of disease; 95% confidence intervals (CI) were calculated to estimate the precision of the RR. Unless otherwise stated, all RR's presented in the text of this paper represent

unadjusted RR's. Chi-square and Fisher's exact tests were used to compare HTLV-I infected and uninfected children with respect to sex and income categories. Wilcoxon rank-sum test was used to compare the mean clinical observation periods and mean values of hematologic parameters between the HTLV-I infected and uninfected groups. A two-sided alpha value of 0.05 was used as criteria for statistical significance. All statistical analysis was conducted using Statistical Analysis Software (SAS Institute, Cary, N.C.) version 6.03 on a personal computer.

Results

A total of 308 children born to HTLV-I seropositive and seronegative mothers achieved at least one clinic visit and are included in this analysis. These include 181 children born to HTLV-I seropositive mothers, and 127 children born to HTLV-I seronegative mothers. Twenty-eight of the 181 children born to seropositive mothers became infected with HTLV-I at an estimated mean age of 14 months (range: 4 – 27 months). In this analysis of health affects associated with HTLV-I we compared the rates of targeted health outcomes ascertained by prospective clinical follow-up in 28 HTLV-I infected and 280 uninfected children.

HTLV-I infected children were composed of 57.1% males, compared to 48.2% males among HTLV-I uninfected children (P = 0.36) (Table 1). A higher proportion of HTLV-I infected children (35.7%) were born to mothers of low income status, compared to uninfected children (21.0%, P = 0.08). Similar proportions of children in the two groups were born at the two participating antenatal clinics (P = 0.87). The 28 HTLV-I infected children accumulated a mean of 6.7 years of clinical observation and the 280 HTLV-I uninfected children accumulated a mean of 5.5 years of clinical observation

(P = 0.81).

Interim Medical History

Interim medical history measured the occurrence of a child's illness since their preceding clinic visit. HTLV-I infected children had similar rate of asthma as uninfected children (RR = 0.9, CI = 0.3-2.2), although asthma was associated with low income (RR =1.9, CI = 1.0-3.5) (Table 2). Mothers were also queried regarding two bacterial infections: ear infection and pneumonia. HTLV-I infected children developed fewer ear infections than uninfected children (RR = 0.3, CI = 0.1-1.1). No child developed pneumonia during the course of this study. HTLV-I infected and uninfected children had a similar rates of rash as an intercurrent illness (RR = 0.8, CI = 0.4-1.4). As expected, HTLV-I infection was not associated with the incidence of diarrhea, which served as a means of assessing observer bias (RR = 0.9, CI = 0.5 – 1.7).

Physical Examination

An examination of skin diseases associated with HTLV-I infection resulted in one case of infective dermatitis that developed in a 46 month old child, 19 months following his estimated time of infection [19]. His skin disease responded to treatment with a topical steroid and oral antibiotic but relapsed intermittently during periods of non-treatment until the end of his clinical observation at 78 months. HTLV-I infected children had a greater than four-fold increased rate of seborrheic dermatitis than HTLV-I uninfected children (RR = 4.2, CI = 1.7-10.2) (Table 2). Among HTLV-I infected children, the mean age at diagnosis was 5.0 years (Table 3). One of these seven infected children with seborrheic dermatitis had a previous diagnosis of infective dermatitis [19].

HTLV-I uninfected children with seborrheic dermatitis were diagnosed at a mean age of four years, which was not statistically different from the age of diagnosis among infected children (P = 0.26). No child was diagnosed with seborrheic dermatitis at more than two clinic visits.

Unlike children with seborrheic dermatitis, children with eczema were diagnosed at multiple clinic visits. Considering the tendency of eczema to recur, a child was considered to have a diagnosis of eczema if he/she had a minimum of three clinic visits at which eczema was diagnosed. HTLV-I infected children had almost a three-fold higher rate of eczema compared to HTLV-I uninfected children (RR = 2.9, CI = 1.8-4.9) (Table 2). There was considerable overlap among the children with diagnoses of seborrheic dermatitis and eczema. Each of the seven HTLV-I infected children with seborrheic dermatitis also had a diagnosis of eczema.

Neurologic examination included an assessment of reflexes, muscle tone, power, and ambulation at clinic visits occurring from 30 months to ten years of age. Lower body reflexes were assessed as normal, hyporeflexic or hyperreflexic. Persistent hyperreflexia was defined as having at least two clinic visits at which hyperreflexia was detected. HTLV-I infected children had more than a three-fold higher rate of persistent hyperreflexia of the lower limbs (RR = 3.6, CI = 1.6-8.1) (Table 2). In the analysis of increased muscle tone, decreased muscle tone was not considered. The rate of increased muscle tone was higher among HTLV-I infected children than uninfected children, but few infected children had this outcome (RR = 2.2, CI = 0.4-10.7). Muscle power was normal for all HTLV-I infected children. The rate of gait abnormality was lower in HTLV-I infected children compared to uninfected children (RR = 0.4, CI = 0.5-3.4)

Lymphadenopathy was defined as the detection of lymph nodes ≥ one centimeter in size at a minimum of two lymph node sites during a single clinic visit. Lymph node sites included epitrochlear, occipital, preauricular, anterior and posterior cervical, supraclavicular, axillary and inguinal sites. The incidence rate of lymphadenopathy was 70% higher among HTLV-I infected children than uninfected children, although this association was of borderline statistical significance (RR = 1.7, CI = 10-2.9) (Table 2). General physical examination also included an assessment of cardiac abnormality. Examination of cardiac abnormality was limited to heart murmur in this study. Because heart murmur is highly prevalent in children under the age of two years, analysis was restricted to children ages 30 months and older. HTLV-I infected children had a similar rate of heart murmur as HTLV-I uninfected children (RR = 1.5, CI = 0.7-3.1).

Body mass index (BMI) was used as an indicator of growth. HTLV-I infection in girls was not associated with a low BMI in the unadjusted analysis (RR = 1.2, CI = 0.4-3.5) (Table 2). However, HTLV-I infected girls had a two-fold increased rate of a low BMI measurement after adjustment for income, although it was not statistically significant (RR = 2.0, CI = 0.6-6.5) (Table 2). Median BMI values were examined within four age-specified strata (2.5 – 3.0 years, 5.0 – 5.5 years, 8.0 – 8.5 years, 9.0 – 10.0 years). Within the 8.0 to 8.5 year strata, HTLV-I infected girls had a lower median BMI level than uninfected girls (14.7 vs. 16.1, P = 0.06). Median values were similar between infected and uninfected girls in the three other age-strata (P = 0.69, P = 0.67, P = 0.25, respectively). Among boys, HTLV-I infection was not associated with a low BMI measurement (RR = 1.0, CI = 0.4-2.7). Median BMI values were similar between HTLV-I infected and uninfected boys in the three age strata examined (2.5 – 3.0 years,

P = 0.50; 5.0 – 8.5 years, P = 0.39; 9.0 – 10.0 years, P = 0.39).

Hematologic factors

The rates of several hematologic abnormalities were higher in HTLV-I infected children than uninfected children. HTLV-I infected children had a 2.5-fold higher rate of severe anemia than uninfected children (RR = 2.5, CI = 0.8-7.9), although these two groups had the same median hemoglobin levels (11.4 g/dl) (Table 2). Examination of available laboratory records for 15 of the 17 HTLV-I infected and uninfected children with severely low hemoglobin indicated that ten of these children had hypochromic microcytic anemia. The mean ages of diagnosis of severe anemia were similar for infected and uninfected children (4.3 years, 4.0 years, respectively (P = 0.42, Table 3). A greater proportion of HTLV-I infected children had multiple clinic visits at which severe anemia was diagnosed than uninfected children (75.0% vs 39.0%, respectively), although this difference was not statistically significant (P = 0.24).

An assessment of other laboratory values targeted eosinophil count, white blood cell count (WBC), lymphocyte count and abnormal lymphocyte percent. HTLV-I infected children had a decreased rate of developing eosinophilia, which was of borderline statistical significance (RR = 0.6, CI = 0.3-1.2) (Table 2). This association reflected a lower mean eosinophil count (301, CI = 221-386), among infected children compared to uninfected children (404, CI = 364-848), (P = 0.06). HTLV-I infected children had a similar rate of leukocytosis as uninfected children (RR = 0.9, CI = 0.2-3.8) and lymphocytosis (RR = 1.1, CI = 0.2-4.8) as uninfected children.

Varying frequencies of lymphocytes having abnormal morphology were examined for association with HTLV-I status. HTLV-I infected and uninfected children had similar rates of abnormal lymphocytes detected at a frequency of 1.0% of all lymphocytes (RR = 1.1, CI = 0.7-1.9). HTLV-I infected children had a 44.0% increased rate of abnormal lymphocytes at a frequency of $\geq$2.0% compared to uninfected children (RR = 1.4, CI = 0.8-2.7). Similarly, HTLV-I infected children had less than a two-fold higher rate of abnormal lymphocytes detected at a higher frequency ($\geq$ 3.0%) (RR = 1.8, CI = 0.7-4.9) (Table 2). The most extreme frequency of abnormal lymphocytes detected was 5.0%.

In summary, HTLV-I infected children had significantly higher incidence rates of eczema, seborrheic dermatitis, lymphadenopathy and persistent hyperreflexia compared to uninfected children. Additionally, infected children had a higher incidence rate of severe anemia compared to uninfected children, which was of borderline statistical significance. Among HTLV-I infected children the mean ages of diagnosis for these health outcomes ranged from 4.2 years of age for eczema to 5.0 years of age for seborrheic dermatitis and were similar to those of uninfected children (Table 3).

There was considerable overlap of these health outcomes among HTLV-I infected children (Figure 2). Eighteen of the 28 infected children had a diagnosis of eczema. Sixteen of those 18 children with eczema also had a diagnosis of lymphadenopathy. All seven chidlren with seborrheic dermatitis had diagnoses of both eczema and lymphadenopathy. All four children with a diagnosis of severe anemia had diagnoses of lymphadenopathy and eczema; three of those four also had a diagnosis of seborrheic dermatitis. Seven of the nine children with a diagnosis of persistent hyperreflexia had a

diagnosis of eczema. Four of those seven children with persistent hyperreflexia and eczema also had a diagnosis of seborrheic dermatitis. Two of those four children had an additional diagnosis of severe eczema.

Discussion

HTLV-I infection in early childhood is associated with infective dermatitis, which is characterized as a severe exudative eczema of the scalp, neck, retroauricular, paranasal, groin and axilla regions [16]. Infection with *Staphlococcus aureus and Beta hemolytic streptococcus* bacteria are common features, as are severe anemia, lymphadenopathy, elevated immunoglobulins and elevated CD4:CD8 ratio. Onset of infective dermatitis occurs between ages three and 16 years, with abatement of symptoms in puberty [15]. With the exception of infective dermatitis, HTLV-I associated morbidity has not been well studied in children.

We examined the incidence of morbidity over a ten-year period in a cohort of HTLV-I infected and uninfected children and hypothesized skin diseases which are considered differential diagnoses for infective dermatitis (seborrheic dermatitis and other eczemas) would be associated with HTLV-I. Additionally, we hypothesized that the less severe diseases and conditions associated with HTLV-I in adults would be associated with HTLV-I in children. Although we did not expect to detect any cases of ATL or HAM/TSP, we hypothesized that HTLV-I infected children would have higher incidence rates of some of the signs and symptoms associated with ATL and HAM/TSP. Thus we examined associations of HTLV-I with lymphadenopathy and other signs of ATL, including leukocytosis, lympohocytosis and the presence of abnormal lymphocytes. Additionally we examined associations of HTLV-I with gait abnormality, and other

features of HAM/TSP, including hyperreflexia and increased muscle tone and decreased muscle power. We also examined the incidence rates of anemia, asthma and bacterial infections in these children. Based on a clinical observation that children with infective dermatitis were small for their age, we hypothesized that HTLV-I infected children would have lower body mass index than uninfected children.

Results of this study suggested several HTLV-I-associated health outcomes. Cutaneous findings on physical examination were prominent with HTLV-I infected children having a significant 4.2-fold increased rate of seborrheic dermatitis and a significant 2.9-fold increased rate of eczema. These data are consistent with results obtained from a study of transfusion recipients of HTLV-I infected blood units in Jamaica which reported a non-significantly increased frequency of skin disease detected by physical examination among the recipients who seroconverted [20]. We found no association of HTLV-I with interim history of skin rash. Similarly a study of HTLV-I associated morbidity among Jamaica food service workers which did not conduct skin examinations found no associations between HTLV-I seropositivity and past medical history or recent symptoms of skin rash [13]. Although HTLV-I seropositivity was associated with a history of eczema-like skin diseases among pregnant women in Salvador, Bahia State, Brazil [21].

The association of HTLV-I with skin disease in children was expected based on previous reports. Infective dermatitis of childhood is associated with HTLV-I infection [15,16]. Additionally, Jamaican children with atopic eczema had an HTLV-I prevalence of 14%, which is much higher than the prevalence of < 1.7% among children younger

than ten years of age, suggesting that HTLV-I may be associated with skin diseases that range from mild to severe [16]. Our results support this contention.

The relevance of this finding is based on the link between early childhood infection and development of ATL. There is a high HTLV-I seroprevalence among mothers of patients with ATL, and the higher incidence of ATL among persons infected in childhood than adulthood [22-24]. However, HAM/TSP, generally believed to be associated with adult-acquired infection via sexual- or transfusion-transmission may also be linked to HTLV-I infection acquired in childhood [25,26]. Case reports of five adults with ATL or HAM/TSP included documented childhood histories of infective dermatitis [27-29]. Linking early childhood HTLV-I infection with ATL and HAM/TSP are recent reports of a series of nine children ages one year to sixteen years diagnosed with ATL, and ten children under the age of 18 years diagnosed with HAM/TSP [30,31]. Four of the nine children with ATL and seven of the ten children with HAM/TSP had childhood histories of skin disease [30,31]. Thus HTLV-I-associated skin disease in childhood may be a marker of risk for ATL and HAM/TSP. The low ($\leq$ 4.0%) lifetime risk of developing these diseases among carriers underscores the importance of identifying carriers at increased risk of developing these diseases [23,24].

HAM/TSP is characterized by bladder dysfunction, hyperreflexia, spasticity, difficulty walking and weakness of the lower limbs [10]. In our study HTLV-I infected children had a significant 3.6-fold higher rate of persistent hyperreflexia of the lower limbs than uninfected children. Additionally, infected children had a 2.2-fold higher rate of increased muscle tone of the lower limbs, although this difference was not statistical significant. HTLV-I infected children did not have a higher rate of gait abnormality.

HAM/TSP, manifesting as generalized hyperreflexia and ankle clonus has been reported in a 14 year old child who was followed prospectively since diagnosis with infective dermatitis at two years of age [29]. Another patient diagnosed with infective dermatitis at ten years of age developed HAM/TSP 25 years later, with onset marked by hyperreflexia and hypertonic lower limbs [29]. These two cases were diagnosed with infective dermatitis by Sweet and Walshe in 1966 and 1967, prior to the discovery of HTLV-I, thus providing the longest follow-up of HTLV-I infected children with infective dermatitis to date [32,33]. Continued follow-up of our cohort is planned in order to assess evidence of progression of spasticitiy in children with persistent hyperreflexia or increased muscle tone.

HTLV-I infected children had almost a 70% higher rate of lymphadenopathy compared to HTLV-I uninfected children, which was of borderline statistical significance. Lymphadenopathy is a feature of ATL. Ten of the 18 infected children with lymphadenopathy in this study also had elevated WBC counts, another feature of ATL. One of these children developed lymphadenopathy and an elevated WBC count prior to being diagnosed with both infective dermatitis and seborrheic dermatitis [19], while all children with lymphadenopathy also had diagnoses of seborrheic dermatitis. Lymphadenopathy was described in three of the nine reported cases of pediatric ATL who ranged in age from 18 months to six years of age at the time of diagnosis with ATL [34,35]. Further clinical follow-up of these children will clarify the association of pediatric lymphadenopathy in HTLV-I-associated disease progression.

HTLV-I infected children had almost a 2.5-fold higher rate of severe anemia compared to uninfected children, which was of borderline statistical significance. These

data support an association in children that has been reported in adult HTLV-I carriers in Jamaica and Japan as well as Japanese immigrants to Hawaii [13,12,36]. The severe anemia in a majority of these children was documented as hypochromic microcytic anemia, consistent with iron deficiency. Anemia of iron deficiency was detected in the child who developed infective dermatitis approximately three and nine months sebsequent to infection, but 11 to 16 months prior to developing infective dermatitis [19]. Anemia of iron deficiency was also detected by Sweet and Walshe in Jamaican children with infective dermatitis diagnosed in the 1960's, prior to the discovery of HTLV-I [32,33]. Severe anemia may be due to nutritional deficiency or parasitic infection. Children with infective dermatitis were reported to have severe anemia as well as abnormally low albumin levels compared to atopic children, supporting a role for nutrition in severe anemia [16]. However 44.0% of those children with infective dermatitis also had parasites detected by stool microscopy, including A*scaris*, *Ancylostoma*, and *Trichuris* [16]. Thus, the pathogenesis of severe anemia in childhood HTLV-I infection is uncertain.

There was considerable overlap in diagnoses of eczema, seborrheic dermatitis, lympadenopathy, severe anemia and persistent hyperreflexia among HTLV-I infected children. The mean ages of diagnoses with these conditions ranged from 4.2 years for eczema to 5.0 years for seborrheic dermatitis. Seborrheic dermatitis may be the most obvious marker of myriad abnormalities associated with HTLV-I.

Other hematologic abnormalities associated with HTLV-I in these children involve eosinophil count and abnormal lymphocytes. HTLV-I infected children had a 40% lower rate of eosinophilia and a lower median eosinophil count than uninfected children, both

of which were of borderline statistical significance. A significantly decreased risk of eosinophilia has been reported for adult HTLV-I carriers in Jamaica and Japan [13,14]. In adults, a low frequency of eosinophilia may explain the increased intensity of strongyloides infestation among HTLV-I carriers, as eosinophils are important in the immune response to this parasitic infection [37,38]. LaGrenade, et al. reported that children with HTLV-I-associated infective dermatitis had a higher frequency of parasitic infections and a significantly lower eosinophil count than children with atopic eczema, although these differences were not significant [16]. An examination of the association of pediatric HTLV-I infection and eosinophilia warrents further study with a larger sample size.

HTLV-I infected children had an 80% higher rate of abnormal lymphocytes at a frequency of $\geq$ 3.0% compared to uninfected children, although this association was of borderline statistical significance. Abnormal lymphocytes at a higher frequency (6.0%) were detected in 30.0% of adult HTLV-I carriers in Miyazaki, Japan, and were associated with increased HTLV-I proviral load, increased lymphocyte count, increased activated (CD25+) T-cells, increased CD4:CD8 ratio, decreased serum albumin and decreased eosinophils [12,9,14]. The association of abnormal lymphocytes with increased HTLV-I proviral load and activated T-lymphocytes suggested that abnormal lymphocytes may be HTLV-I infected lymphocytes. It is possible that HTLV-I infected children with $\geq$ 3.0% abnormal lymphocytes have an increased proviral load and therefore are more likely to develop HTLV-I-associated disease. The one child in our cohort who was diagnosed with both infective dermatitis and seborrheic dermatitis had a high proviral load 12 months post-infection, which increased over time, and a 2.2% frequency of abnormal

lymphocytes two years subsequent to development of infective dermatitis [19]. Further analysis of HTLV-I proviral load in association with this health outcome is described in a companion paper [Maloney EM. HTLV-I proviral load is associated with development of seborrheic dermatitis in Jamaican children].

HTLV-I infected children did not have an elevated risk of asthma in our study. History of asthma was associated with HTLV-I seropositivity among men in a cohort study of Japanese adults, but not among Jamaican adults or U.S. blood donors [11,13,18]. The association among male but not female Japanese adults was explained as possibly due to the potentially longer duration of infection among males, as opposed to females who are at greater risk of acquiring HTLV-I infection sexually [13,40]. If this is so, the short duration of infection among Jamaican children could have precluded our ability to detect an association of HTLV-I with asthma in our study.

In summary, we examined the incidence of morbidity in a cohort of HTLV-I infected and uninfected children who were clinically observed from six weeks to a maximum of ten years of age. The blinding of the pediatrician to the HTLV-I status of each child seems to have been effective, as the incidence of diarrhea, known to be unrelated to HTLV-I status was not higher among infected children compared to uninfected children. The value of this study is based on the unique existence of this pediatric cohort with extended clinical observation. However, a limitation of our study was the relatively small number of HTLV-I infected children which may have limited the power of this study to detect statistically significant associations between HTLV-I infection and health outcomes.

In our study HTLV-I infected children had significantly higher rates of seborrheic dermatitis and eczema than uninfected children, possibly expanding the range of HTLV-I associated skin diseases in children. HTLV-I infected children also had significantly higher incidence rates of persistent hyperreflexia, a clinical feature of HAM/TSP. Additionally, infected children had non-significantly elevated rates of health outcomes reported in association with HTLV-I seropositivity in adults: severe anemia, lymphadenopathy, presence of abnormal lymphocytes and decreased eosinophilia. Further prospective clinical observation of this cohort is planned to further examine the extended natural history of HTLV-I associated disease in children.

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Table 1. Distribution of demographic factors among HTLV-I infected and HTLV-I uninfected Jamaican Children

Factor	HTLV-I Infected (n = 28)	HTLV-I Uninfected (n = 280)	P-value
Sex			
Male	16 (57.1)	135 (48.2)	P = 0.36
Female	12	145	
Maternal income[a]			
Low (< $100J/week)	10 (35.7)	58 (21.0)	P = 0.08
High (≥ $100J/week)	18	218	
Hospital Clinic			
Jubilee	22 (78.6)	229 (81.8)	P = 0.87
UWI	6	51	
Duration of follow-up (years)			
Mean	6.7	5.5	P = 0.81
95% C.I.	(5.6 – 7.8)	(5.1 – 5.9)	

( ) = percent;

$100J = 100 Jamaican dollars (at 1989 exchange rate of $5.50J = $1.00 U.S.);

[a] Information on income was missing for four HTLV-I seronegative mothers;

UWI = University of the West Indies;

C.I. = confidence interval.

Table 2. Distribution of health outcomes among HTLV-I infected and uninfected Jamaican children, and associated rate ratios

Health Outcome	HTLV-I Infected (n = 28)	HTLV-I Uninfected (n = 280)	RR	95% CI	Adj.[a] RR	95% CI
Skin assessment						
Seborrheic dermatitis	7	18	4.2	(1.7-10.2)	4.5	(1.8-11.2)
Eczema	18	82	2.9	(1.8-4.9)	3.1	(1.8-5.3)
Neurologic assessment[b]						
Persistent Hyperreflexia	9	19	3.6	(1.6-8.1)	3.9	(1.7-8.9)
Increased muscle tone	2	6	2.2	(0.4-10.7)	2.2	(0.4-12.7)
Gait abnormality	1	15	0.4	(0.1-3.4)	0.5	(0.7-3.9)
General physical examination						
Lymphadenopathy	18	97	1.7	(1.0-2.9)	1.7	(0.9- 2.8)
Heart murmur	8	47	1.5	(0.7-3.1)	1.4	(0.7-3.1)
Low body mass index[c]						
Girls	4	8	1.2	(0.4-3.5)	2.0	(0.6 - 6.5)
Boys	5	27	1.0	(0.4-2.7)	0.9	(0.4 - 2.6)
Hematologic factors[d]						
Severe anemia[e]	4	13	2.5	(0.8-7.9)	2.8	(0.8 - 9.2)
Eosinophilia[f]	8	87	0.6	(0.3-1.2)	0.6	(0.3 - 1.2)
Elevated white blood cell count (WBC)[g]	2	16	0.9	(0.2-3.8)	1.0	(0.2 - 4.5)
Elevated lymphocyte count[h]	2	13	1.1	(0.2-4.8)	1.5	(0.3 - 7.1)
Abnormal lymphocytes (3.0%)	5	20	1.8	(0.7-4.9)	2.2	(0.8-6.1)

Interval Health History

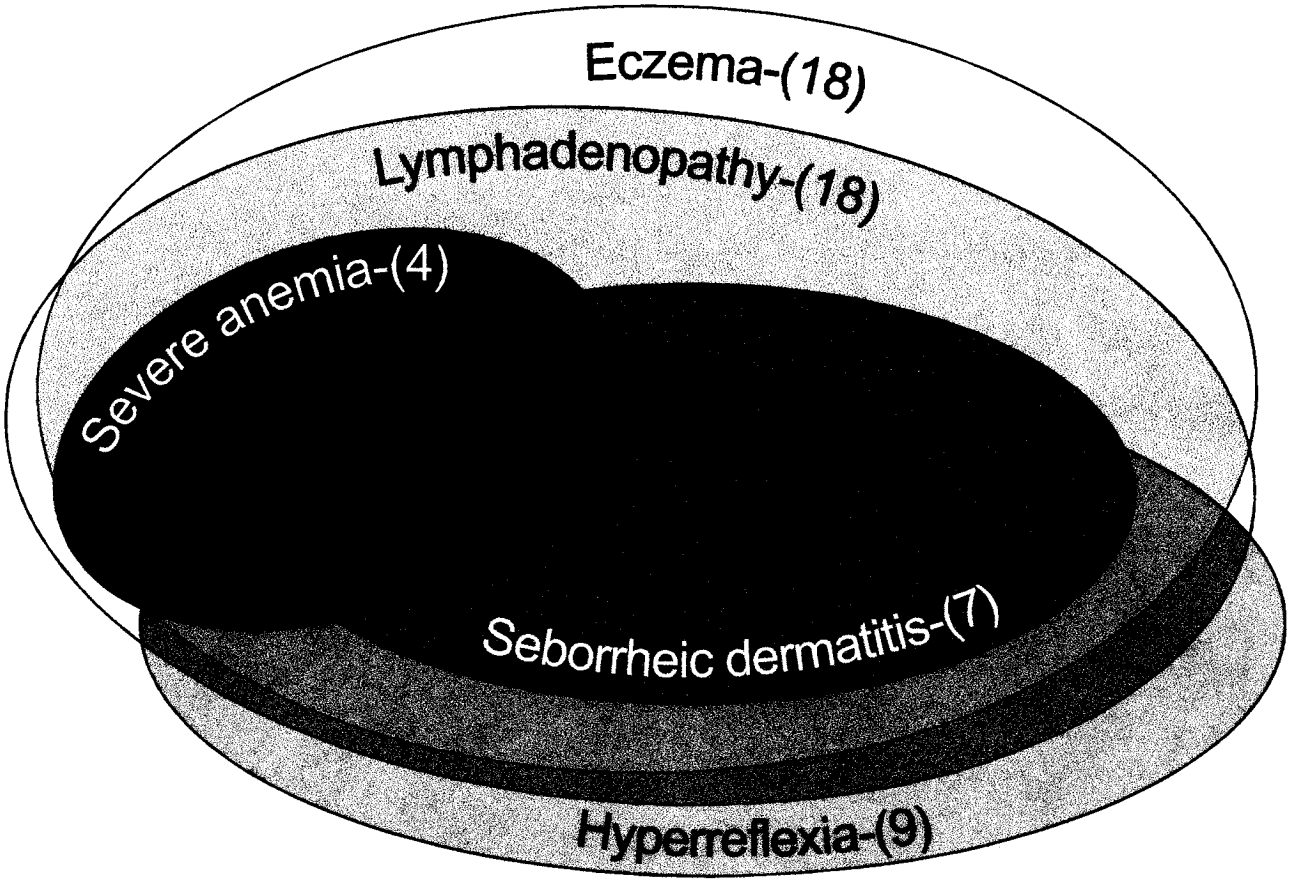
Asthma	5	51	0.9	(0.3-2.2)	0.7	(0.3-1.9)
Ear infection	2	26	0.3	(0.1-1.1)	0.3	(0.1-1.1)
Pneumonia	0	0	---	---	---	---

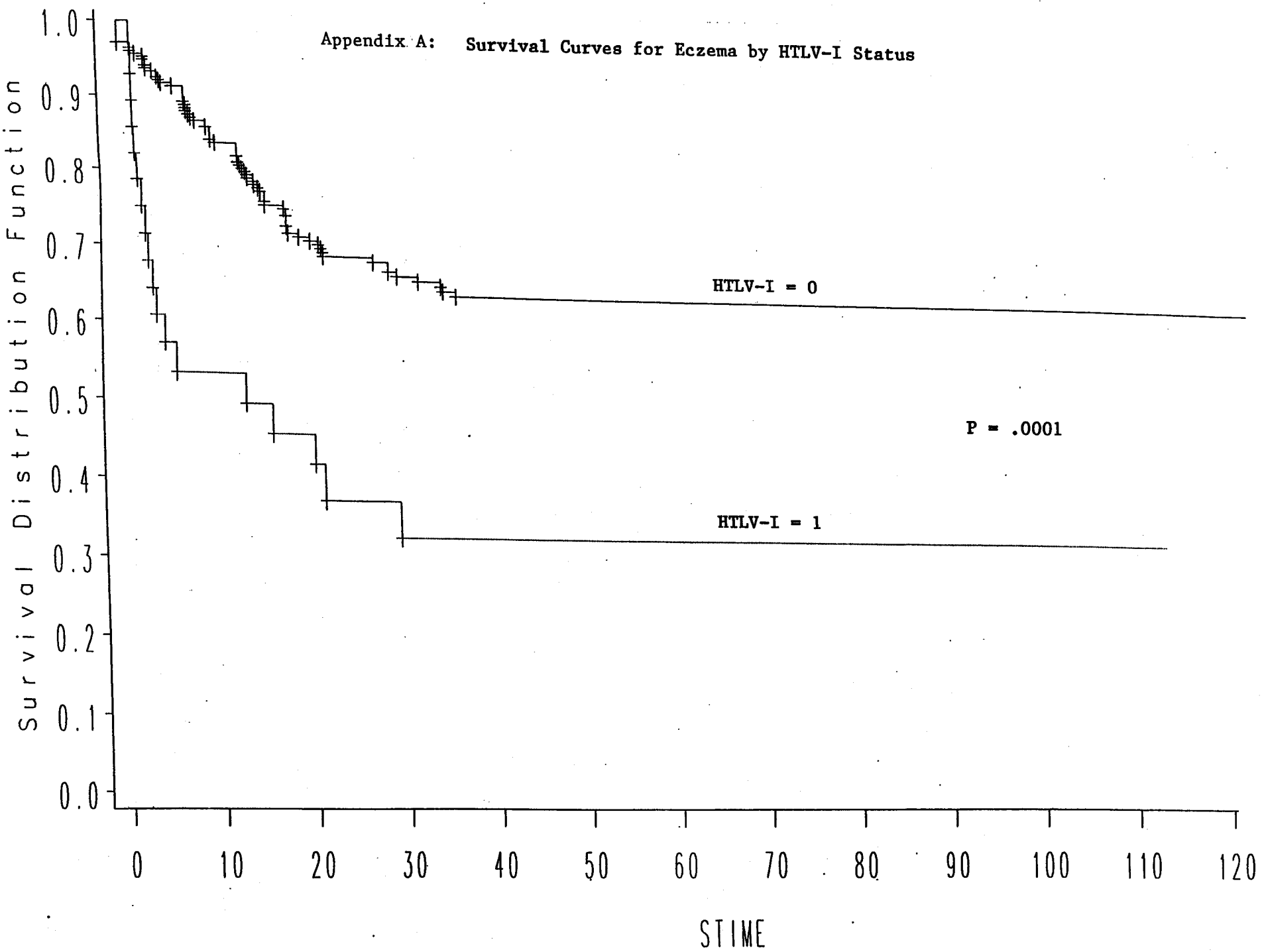
RR = rate ratio; CI = confidence interval;
[a] adjusted for sex and maternal income;
[b,d] the total number of children assessed for asthma, neurologic and hematologic factors include 26 HTLV-I infected and 189 uninfected children;
[c] Girls: BMI <14.0 (ages 30 - 36 months); <13.8 (ages 42 - 54 months); <13.6 (ages 60 - 66 months); <13.4 (ages 72 - 90 months); <13.8 (ages 96 - 114 months); <14.0 (ages 120 months); Boys BMI <14.4 (ages 30 - 36 months); <14.2 (age 42 months); <14.0 (ages 48 - 54 months); < 13.8 (ages 60 - 102 months); <14.0 (ages 108 - 114 months); <14.2 (age 120 months);
[e] Hemoglobin < 9.0 g/dl (ages 30 - 36 months); < 9.6 g/dl (ages 42 - 66 months) and < 10.0 g/dl (ages 72 - 120 months);
[f] Eosinophils > 800;
[g] WBC > 15.5 $X10^3$ (ages 30 - 66 months) and > 8 X 10^3 (ages 72 - 114 months) and >13.5 X 10^3 (age 120 months).
[h] lymphocyte count > 8,500 (ages 30 - 120 mos.).

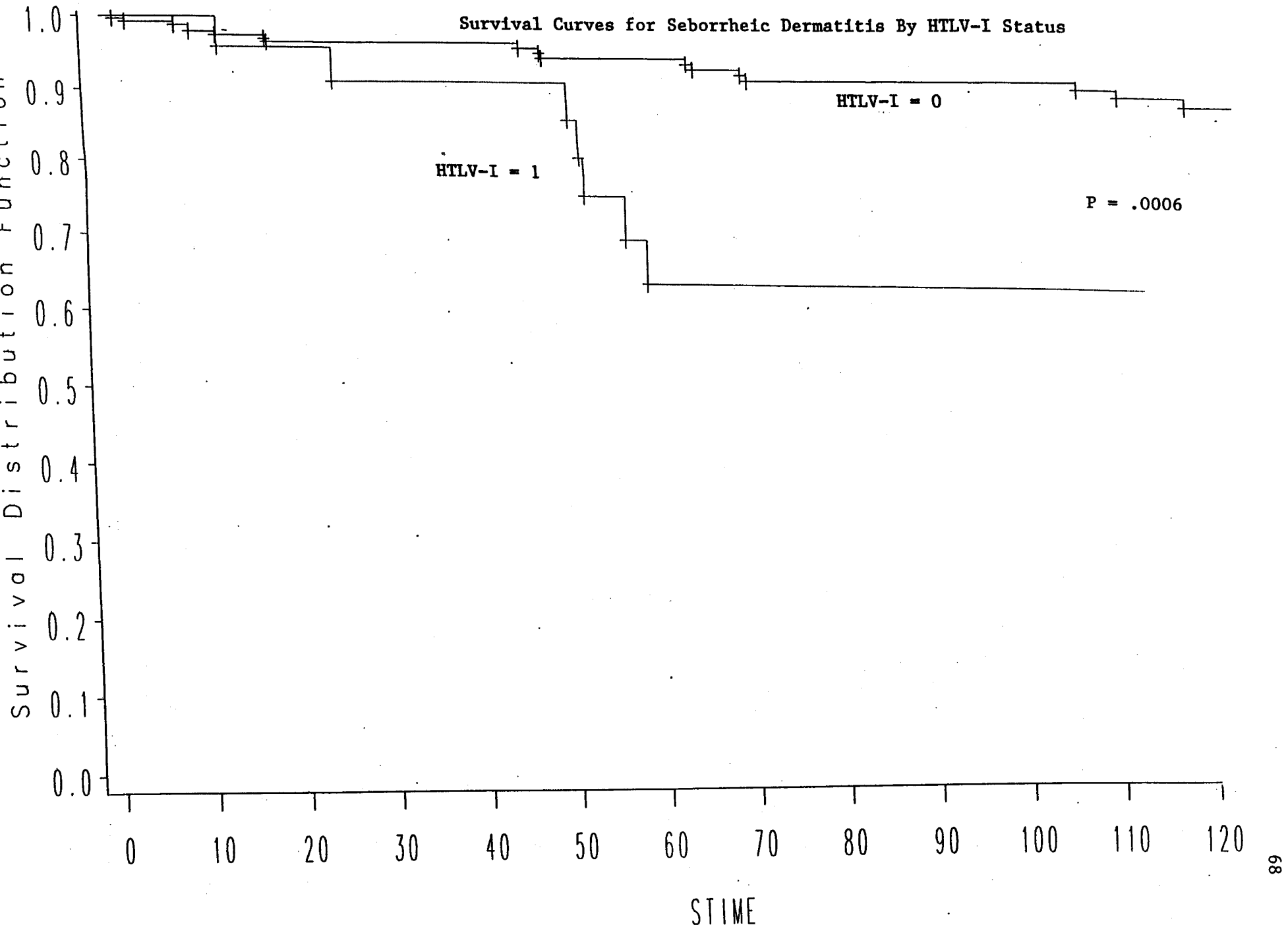
Table 3. Mean ages of diagnosis with HTLV-I associated health outcomes, by HTLV-I status

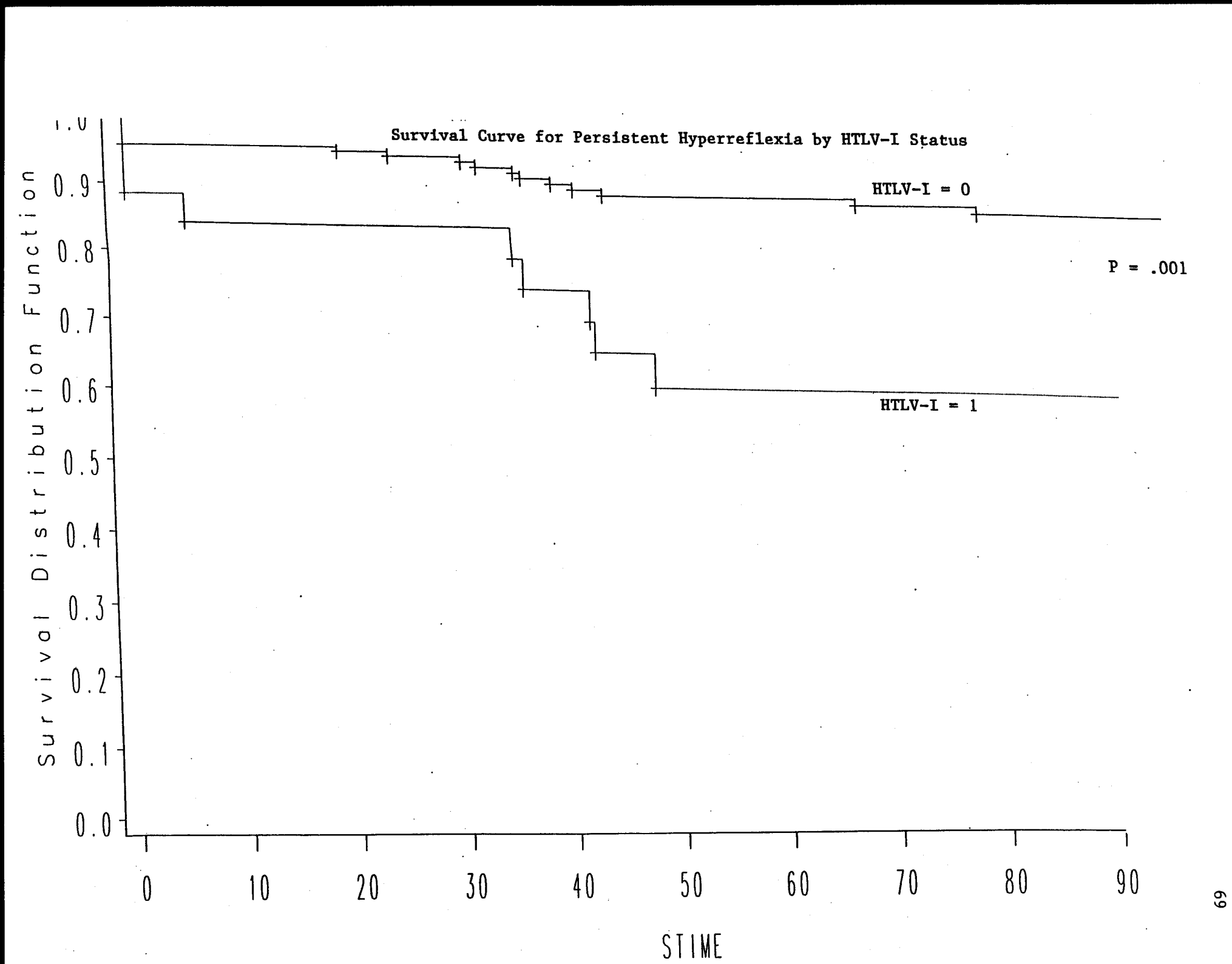
Health Outcome	HTLV-I Infected	HTLV-I Uninfected	P-value
Seborhheic Dermatitis	5.0 (3.9 – 6.0)	4.0 (2.5 – 5.5)	P = 0.26
Eczema	4.2 (3.2 – 5.2)	4.0 (3.4 – 4.5)	P = 0.58
Severe Anemia	4.3 (0.8 – 7.9)	4.0 (2.6 – 5.4)	P = 0.42
Lymphadenopathy	4.7 (3.3 – 5.9)	3.6 (3.1 – 4.0)	P = 0.77
Persistent Hyperreflexia	4.5 (3.3 – 5.7)	4.6 (3.7 – 5.5)	P = 0.80

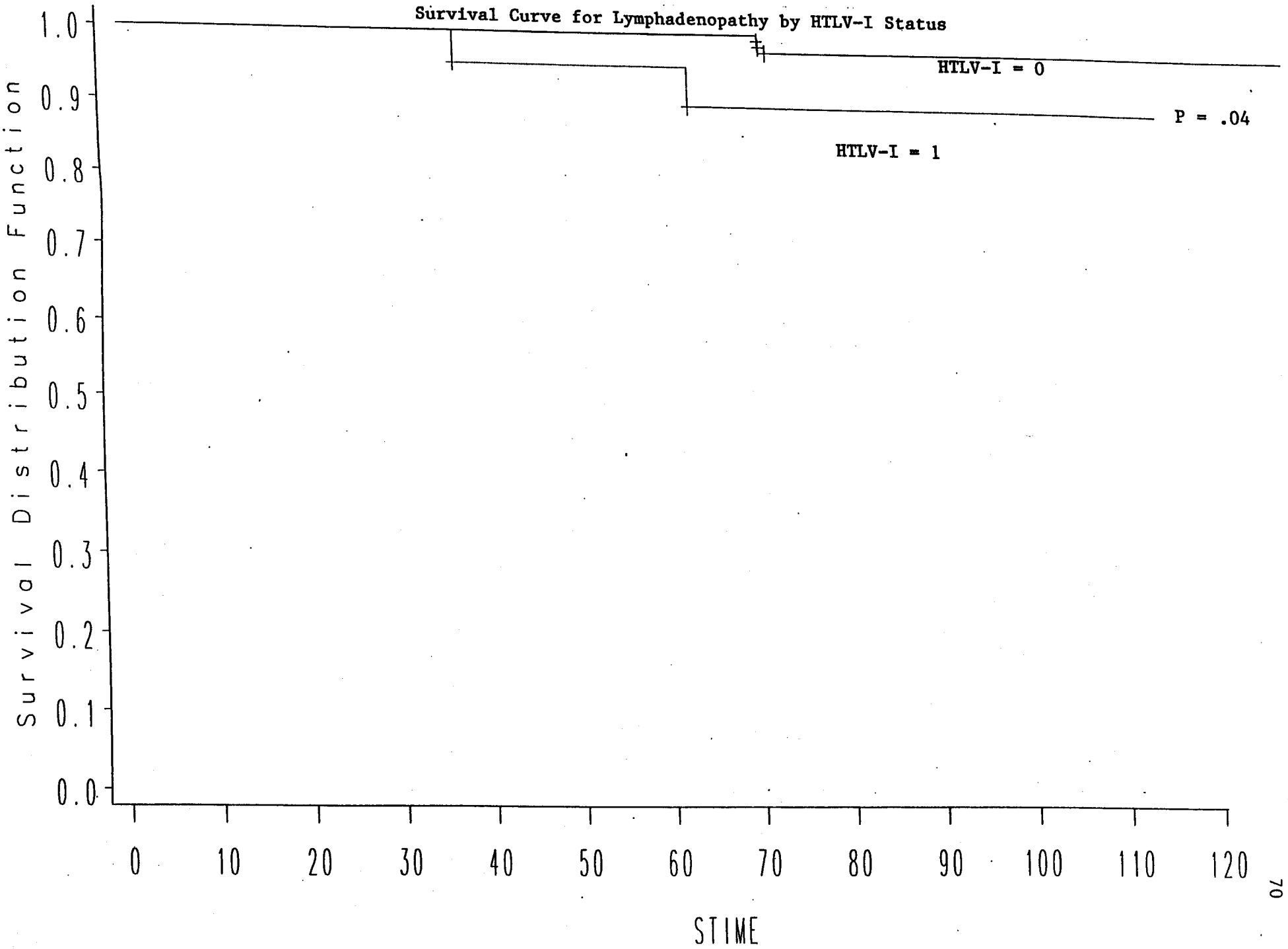
HTLV-I Infected Children (n=28)

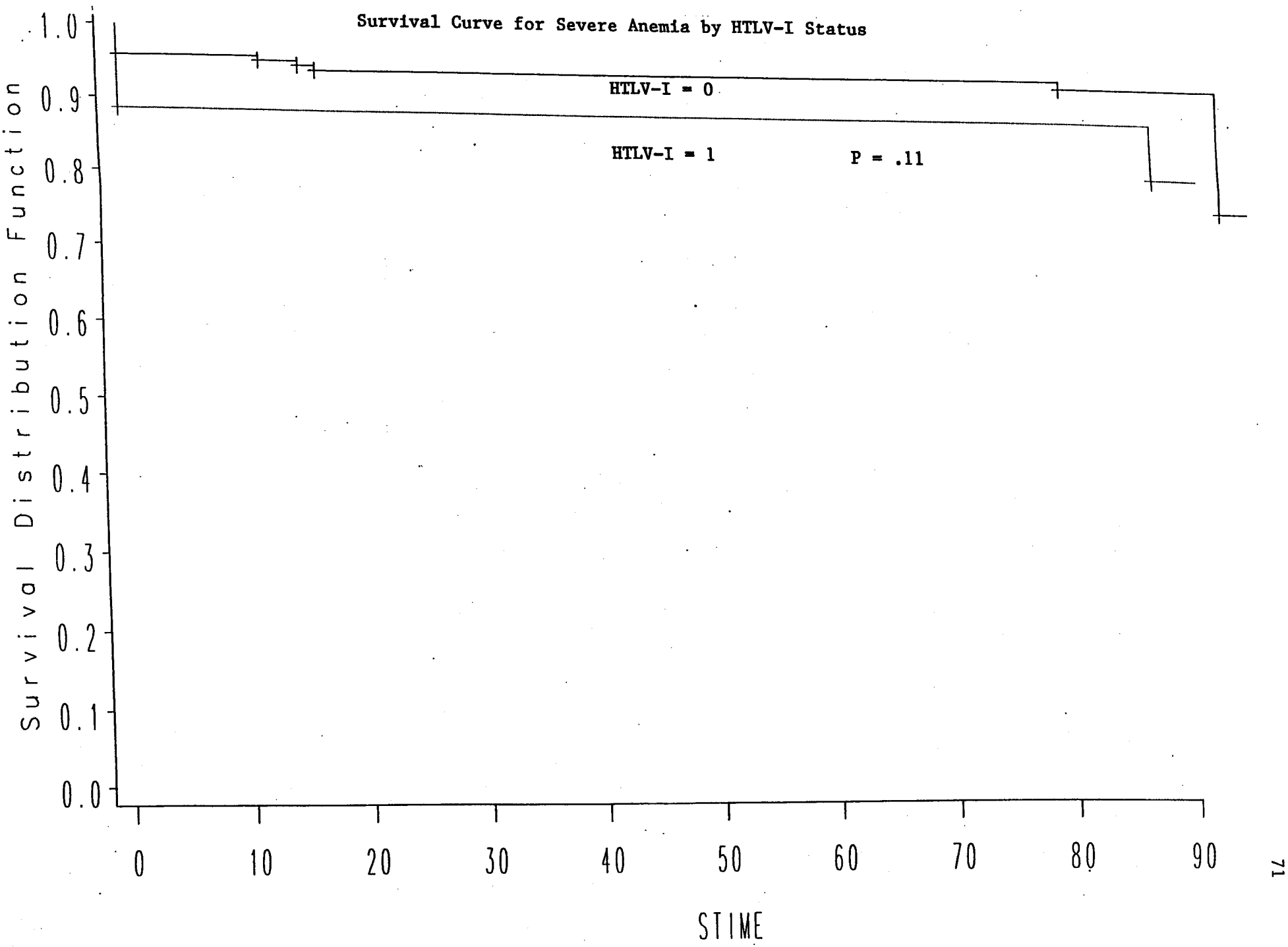


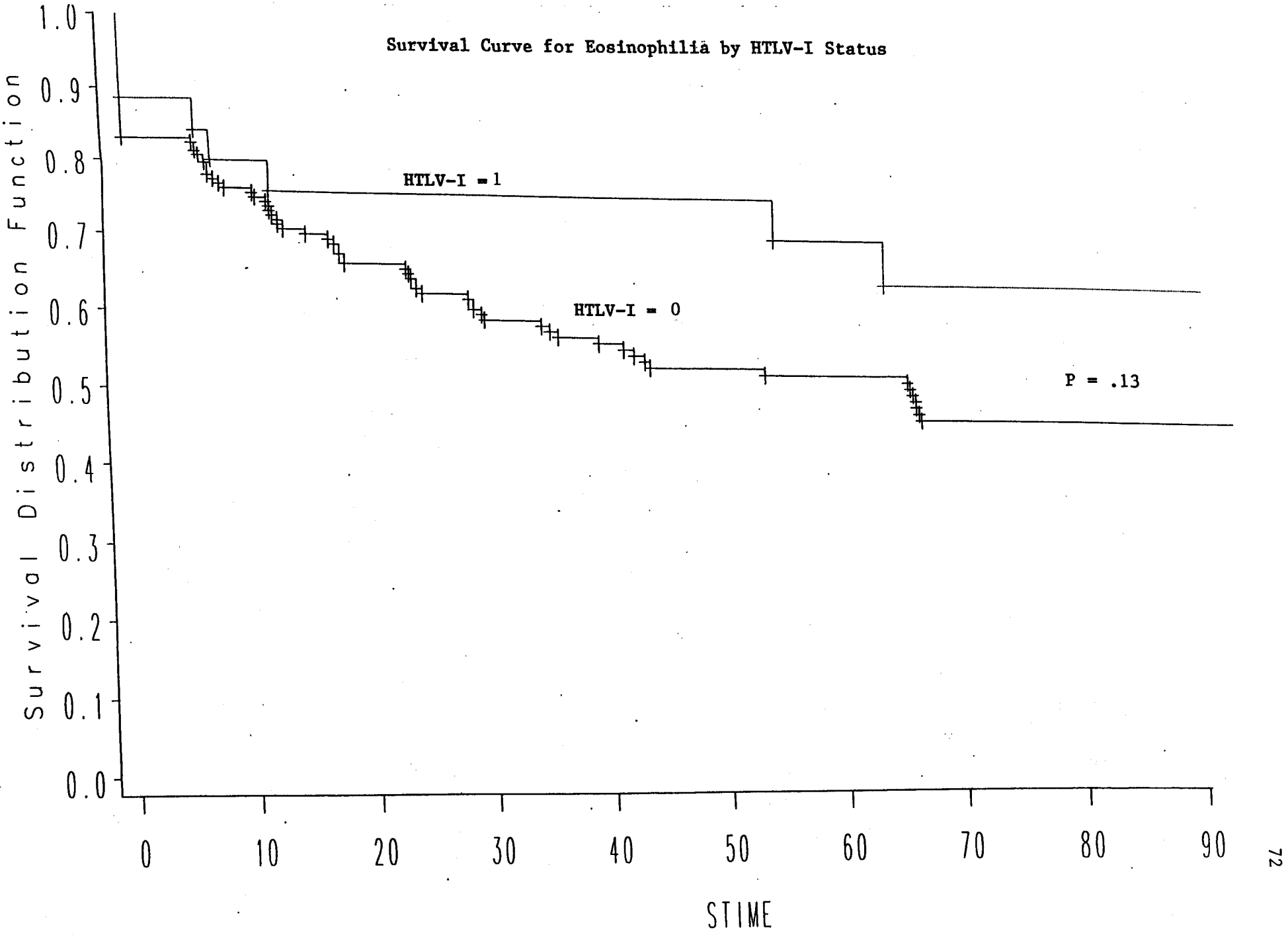

Appendix A: Survival Curves for Eczema by HTLV-I Status
Survival Distribution Function
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
HTLV-I = 0
P = .0001
HTLV-I = 1
0
10
20
30
40
50
60
70
80
90
100
110
120
STIME

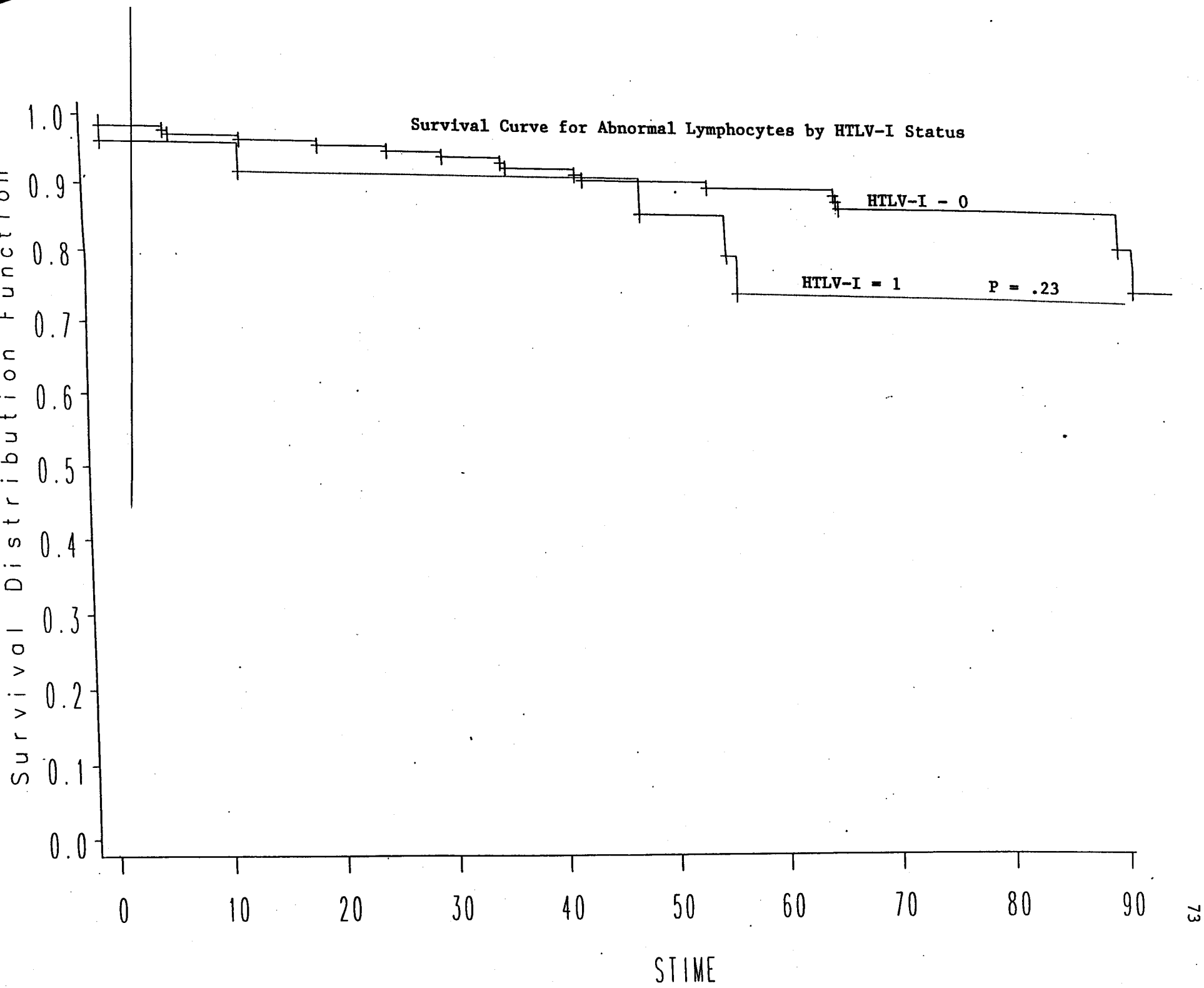

Survival Curves for Seborrheic Dermatitis By HTLV-I Status
HTLV-I = 0
HTLV-I = 1
P = .0006
Survival Distribution Function
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
10
20
30
40
50
60
70
80
90
100
110
120
STIME


Survival Curve for Persistent Hyperreflexia by HTLV-I Status
HTLV-I = 0
HTLV-I = 1
P = .001
Survival Distribution Function
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
10
20
30
40
50
60
70
80
90
STIME


Survival Curve for Lymphadenopathy by HTLV-I Status
HTLV-I = 0
P = .04
HTLV-I = 1
Survival Distribution Function
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
10
20
30
40
50
60
70
80
90
100
110
120
STIME


Survival Curve for Severe Anemia by HTLV-I Status
HTLV-I = 0
HTLV-I = 1
P = .11
Survival Distribution Function
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
10
20
30
40
50
60
70
80
90
STIME


Survival Curve for Eosinophilia by HTLV-I Status
Survival Distribution Function
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
HTLV-I = 1
HTLV-I = 0
P = .13
0
10
20
30
40
50
60
70
80
90
STIME


Survival Curve for Abnormal Lymphocytes by HTLV-I Status
HTLV-I - 0
HTLV-I = 1
P = .23
Survival Distribution Function
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
10
20
30
40
50
60
70
80
90
STIME

Second Manuscript

HTLV-I Proviral Load is Associated with Development of Seborrheic Dermatitis in Jamaican Children

Elizabeth M. Maloney

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Abstract

HTLV-I infection in early childhood is associated with infective dermatitis which occurs at approximately two years of age, and has a relapsing course until abatement in puberty. In a companion paper we described other diseases and conditions that were associated with HTLV-I infection in a cohort of 28 infected and 280 uninfected children who were followed clinically from six weeks to ten years of age. HTLV-I infection was associated with significantly higher incidence rates of seborrheic dermatitis, eczema and persistent hyperreflexia. Additionally, HTLV-I infection was associated with non-significantly increased incidence rates of lymphadenopathy, severe anemia, abnormal lymphocytes, and a decreased rate of eosinophilia. In the current study we examined several viral and immunologic markers, as well as host genetic markers in all 28 HTLV-I infected children from that cohort to assess their associations with development of these targeted health outcomes. Viral markers included HTLV-I proviral load, antibody titer and tax-specific antibody. Immunologic markers included serum levels of several T-helper type-1 and -2 (TH-1 and TH-2) cytokines. Host genetic markers included human leukocyte antigen (HLA) Class II alleles DRB1*1101, DQB1*0301, and DQB1*0602. The geometric mean levels of HTLV-I proviral load were higher among children with seborrheic dermatitis and children with severe anemia, compared to children without these diagnoses. These associations were of borderline statitistical significance. These data support an association of HTLV-I with seborrheic dermatitis and severe anemia in childhood.

Introduction

Human T-lymphotropic virus type I (HTLV-I) is associated with a rare T-cell malignancy called adult T-cell leukemia/lymphoma (ATL) and a chronic demyelinating neurologic disease, called HTLV-I associated myelopathy, or tropical spastic paraparesis (HAM/TSP) [1-3]. More recently, HTLV-I was associated with an inflammatory eye disease, HTLV-I associated uveitis [4]. These diseases are associated with elevated levels of HTLV-I proviral load and antibody titer measured at the time of diagnosis [5-7]. In contrast, HTLV-I tax-specific antibody is present in 94% of HAM/TSP patients but only 60% of ATL patients, and in 48% of population carriers [8,9]. ATL, HAM/TSP and uveitis occur predominantly in the fifth decade of life.

Among children, HTLV-I has been associated with a severe exudative eczema called HTLV-I associated infective dermatitis [10]. In a companion paper, we described several additional HTLV-I associated diseases and conditions that developed in a cohort of children who were observed clinically from six weeks to a maximum age of 10 years [Maloney EM. A cohort study of health effects of HTLV-I infection in Jamaican children]. Twenty-eight of those children became infected and, compared to HTLV-I uninfected children in this cohort, infected children had significantly higher incidence rates of seborrheic dermatitis, eczema and persistent hyperreflexia, and non-significantly higher rates of severe anemia, lymphadenopathy and abnormal lymphocytes. Infected children also had a decreased rate of eosinophilia that was of borderline statitsical significance. The present study examines the pre-diagnostic specimens obtained from the 28 HTLV-I infected children to determine if these health outcomes were associated with HTLV-I viral markers and immunologic markers, as well as several human leukocyte

antigen (HLA) Class II alleles reported to be associated with ATL, HAM/TSP or infective dermatitis.

Based on elevated levels of HTLV-I proviral load and antibody titer associated with ATL and HAM/TSP [5-6], we hypothesized that children with diagnoses of seborrheic dermatitis, eczema, persistent hyperreflexia, severe anemia, lymphadenopathy, abnormal lymphocytes and those without a diagnosis of eosinophilia would have higher levels of HTLV-I proviral load and antibody titer than children without these health outcomes. Based on the high correlation between tax-specific antibody and proviral load [11], we hypothesized that a higher proportion of tax specific antibodies would be detected in children with the targeted HTLV-I associated health outcomes than children without those outcomes. Based on studies of pediatric HIV infection which support associations between elevated levels of interleukin 4 (IL-4) and interleukin 10 (IL-10), which are TH-2 cytokines, and progression to AIDS [12,13], we hypothesized that children with the targetted health outcomes would have a TH-2 cytokine pattern. Additionally, based on the association of HLA Class II haplotype (DRB1*DQB1*(1101-0301)) with HAM/TSP [14], and alleles DRB1*1501-DQB1*0602 with ATL [15,16] we hypothesized that HTLV-I associated health outcomes in our infected cohort would be associated with DRB1*1101, or DQB1*0301, or DQB1*0602.

Materials and Methods

Study Population

This study is composed of the 28 HTLV-I infected children who were born to 212 HTLV-I seropositive women who enrolled in a prospective study of maternal-infant

transmission of HTLV-I between January, 1989 and August, 1990 in Kingston, Jamaica [17]. These children became infected with HTLV-I at a mean estimated age of 14 months. A comprehensive physical examination and phlebotomy were conducted on these children every six weeks for the first six months of life, then every three months up to two years of age and every six months thereafter up to a maximum of ten years of age. These children were breastfed for a mean of 17 months and followed clinically for a mean of 6.7 years (95% confidence interval (CI), 5.6-7.8).

Data Collection

The prospective clinical evaluation of this cohort is described in detail in a companion paper (Maloney EM. A Cohort Study of Health Effects of HTLV-I Infection in Jamaican Children). Briefly, a pediatrician, blinded to each child's HTLV-I status, conducted a general physical examination including skin assessment and phlebotomy at each clinic. Targeted health outcomes for this analysis derived from the physical examination included seborrheic dermatitis, eczema, persistent hyperreflexia and lymphadenopathy. The diagnoses of seborrheic dermatitis and eczema were based on clinical observation. Considering the tendency of eczema to recur, a child was considered to have a diagnosis of eczema if he/she had a minimum of three clinic visits at which eczema was diagnosed. Persistent hyperreflexia was defined as the detection of hyperreflexia at a minimum of two clinic visits. Lymphadenopathy was defined as the presence of lymph nodes of at least one centimeter in size at a minimum of two lymph node sites during a single clinic visit.

Targeted health outcomes derived from complete blood counts (CBC) and blood slides included severe anemia, eosinophilia and abnormal lymphocytes. Severe anemia was defined as hemoglobin (Hgb) levels less than age-specific limits of the normal distribution: Hgb <9.0 g/dl (ages 30 – 36 months); Hgb<9.6 g/dl (ages 42 – 66 months) and Hgb< 10.0 g/dl (ages 72 – 120 months). A review of hematologist's notes was used to determine if anemia was normochromic, normocytic (i.e. probably due to chronic infection or illness) or hypochomic, microcytic (i.e. probably due to iron deficiency). Eosinophilia was defined as eosinophil count $\geq$ 800 per cc of blood. Abnormal lymphocytes are atypical lymphocytes. Analysis is limited to diagnoses of abnormal lymphocytes at a frequency of $\geq$3.0% of peripheral blood mononuclear cells (PBMC), as this frequency was associated with HTLV-I infection in the companion paper [Maloney EM. A Cohort Study of Health Effects of HTLV-I Infection in Jamaican Children].

A trained nurse obtained information on maternal income and duration of breastfeeding from each child's mother. Maternal income was dichotomized as low (< $100 Jamaican/week) and high ($\geq$ $100 Jamaican /week), based on the 1989 exchange rate of $5.50 Jamaican = $1.00 US. Duration of breast feeding was determined as the period between the child's birth and the weaning date reported by the child's mother.

Laboratory

At each clinic visit occurring during the first 24 months whole blood was collected from the children by heel-stick in capillary tubes for HTLV-I serologic and virologic testing. Whole blood was centrifuged and serum and lymphocytes were stored in liquid nitrogen at -70° C until withdrawal for testing. From age 30 months to 120 months, 10cc of heparinized blood was collected and centrifuged and plasma and lymphocytes were

stored as above for the same purposes. Beginning at 30 months and continuing to the end of the study an additional 3.5cc of blood was collected in an EDTA tube to determine complete blood count (CBC) and white blood cell differential, using standard methods. The number of abnormal lymphocytes per 100 peripheral blood mononuclear cells (PBMC) was determined by microscope review of peripheral blood smears. Host DNA used for HLA testing was extracted from one aliquot of lymphocytes per child.

Viral Markers

Analysis of proviral load was applied to lymphocyte specimens obtained from the 28 children approximately 12 months post-infection. HTLV-I proviral load was measured using the ABI PRISM 7700 Sequence Detector [6]. The primer set for HTLV-I pX region was 5N- ACAAAGTTAACCATGCTTATTATCAGC-3N positioned at nt 7276-7302 and 5N-ACACGTAGACTGGGTATCCGAA-3N positioned at nt 7355-7334. The primer set for β-actin was 5N-CACACTGTGCCCATCTACGA-3N positioned at nt 2147-2165 and 5N-CTCAGTGAGGATCTTCATGAGGTAGT-3N positioned at nt 2250-2225. DNA for this purpose was extracted from $1x10^6$ frozen peripheral blood mononuclear cells (PBMC) using PureGene DNA Isolation Kit (Gentra Systems, Minneapolis, MN). Ten μl of DNA solution per sample was analyzed for proviral load in a 96-well format (Perkin Elmer Applied Biosystems, USA). The amount of HTLV-I proviral DNA was calculated by the formula: copy number of HTLV-I (pX) per $1x10^4$ PBMC = {(copy number of pX)/copy number of β-actin/2)} x 10^3. This assay detects as few as one copy per 10^4 PBMC.

HTLV-I antibody titer was analyzed in plasma obtained approximately 12 months post-infection using an whole virus ELISA based on four-fold dilutions of each sample (Genetic Systems EIA). HTLV-I tax specific antibody was measured by ELISA (Tsukuba Eisai Research Laboratories, Tsukuba, Japan) [18].

Immunologic markers

Cytokines were analyzed in serum samples obtained approximately 12 months post-infection using commercial assays (R&D Systems, Minneapolis, MN). Nineteen HTLV-I uninfected children's serum samples obtained at a comparable age were used as laboratory controls. Additionally, among children with seborrheic dermatitis who had available specimens, cytokines were measured at time of diagnosis of seborrheic dermatitis. Selected cytokines included those considered representative of T-helper type 1 (TH-1) immune responses and T-helper type 2 (TH-2) immune responses. TH-1 cytokines included interferon-gamma (IFN-γ), tumor-necrosis factor-alpha (TNF-α) and interleukin-1 alpha (IL-1α) and Interleukin-6 (IL-6). TH-2 cytokines included interleukin-4 (IL-4) and interleukin-10 (IL-10). The lower limits of detection for these cytokines are as follows: IFN-γ: 10 pg/ml; TNF-α (1 pg/ml); IL-1α (1 pg/ml); IL-4 (3 pg/ml); IL-6 (0.7 pg/ml); IL-10 (3 pg/ml).

Host genetic markers

Human leukocyte antigen (HLA) Class II molecular typing was done by single strand conformation polymorphism analysis in combination with PCR sequence-specific primers [19]. DNA for this purpose was extracted by the PureGene DNA isolation kit (Gentra Systems, Inc., Minneapolis, MN).

Statistical Methods

Among these 28 HTLV-I infected children, person-years (PY) at risk for health outcomes determined by physical examination (seborrheic dermatitis, eczema, lymphadenopathy) accumulated from their estimated date of HTLV-I infection to their date of diagnosis with a specific health outcome, their withdrawal from the study, the end of the study period or their date of death. For health outcomes based on hematologic data (severe anemia, eosinophilia, abnormal lymphocytes) or neurologic data (persistent hyperreflexia), PY at risk accrued from the 30 month clinic visit when measurement of these outcomes began, as all 28 children became infected before 30 months. Geometric mean levels of proviral load and antibody titer were compared between children grouped by health outcome status using the Wilcoxon rank-sum test. Fisher's exact test was used to compare the frequency of tax-specific antibody and HLA-Class II alleles between children grouped by health outcome status. Fisher's exact test was also used to compare the proportion of HTLV-I infected children with any elevated cytokines to the proportion of HTLV-I uninfected children with any elevated cytokines (laboratory controls). The Spearman rank correlation coefficient was used to measure the associations of proviral load, antibody titer and presence of tax-specific antibody among the 28 HTLV-I infected children. All statistical analyses were conducted using Statistical Analysis Software (SAS Institute, Cary, NC) version 6.03 on a personal computer.

Results

This cohort of 28 HTLV-I infected children included 16 (57.1%) males and 12 females (42.9%) who were predominantly of a high socioeconomic status (64.3%) as measured by maternal income.

The children's HTLV-I proviral loads measured 12 months post-infection had a geometric mean of 3.6 viral copies per 100 PBMCs (95% CI = 2.3 – 5.7 copies). Children's HTLV-I antibody titers at this same time-point had a geometric mean of 12,221 (95% CI = 5,459 - 27,358) and were significantly correlated with proviral load ($r = 0.57$, $P = 0.002$) (Figure 1). Seventy-five percent of the infected children had tax-specific antibody which was correlated with 'high' (above the median value) levels of both proviral load and antibody titer ($r = 0.58$, $p = 0.001$ for both).

Cytokines were measured in serum samples obtained at the same 12 months post-infection time-point used for testing viral markers. Seven of the 28 children had elevated levels (> 30 pg/ml according to the manufacturer) of at least one cytokine compared to two of 19 HTLV-I pediatric controls ($P = 0.28$) (Table 1). Among HTLV-I infected children T-helper type 1 (TH-1) cytokines accounted for most of the elevated cytokines. Four children had elevated levels of TNF-α and IL-6, one of whom also had elevated IL-1α. This child with elevated levels of all three TH-1 cytokines plus an elevated level of IL-10 (subject no. four) had subsequent diagnoses of seborrheic dermatitis and infective dermatitis. One additional child had elevated levels of TNF-α and IL-1α, as well as elevated levels of a TH-2 cytokine, IL-10. Elevated cytokines in the two uninfected children were restricted to IL-6.

Seven children developed seborrheic dermatitis at a mean age of 5.0 years (95% CI = 3.9 – 6.0). Children who developed seborrheic dermatitis had a higher mean proviral load than children without seborrheic dermatitis, which was of borderline statistical significance (P = 0.08) (Table 2). Children with seborrheic dermatitis also had a higher mean antibody titer than other infected children, although this difference was not statistically significant (P = 0.15). All of the children who developed seborrheic dermatitis had tax-specific antibody, compared with 66.7% of other children (P = 0.14).

In order to assess the cytokine pattern among children newly diagnosed with seborrheic dermatitis, cytokines were measured in five of the seven children who had available samples obtained at the time of diagnosis. All five children had high levels of IL-10 (range: 76 pg/ml – 14,524 pg/ml); two of the five children had elevated levels of IL-6 (700 pg/ml; 7,994 pg/ml) and TNF-" (120 pg/ml, 125 pg/ml) and one child without a detectable level of IL-6 had an elevated level of TNF-" (164 pg/ml). IL-1" and IFN-(were not detected. This cytokine pattern was similar to that of three HTLV-I uninfected children whose sera were tested at the time of diagnosis with seborrheic dermatitis. All three had elevated levels of TNF-" (31 – 503 pg/ml) or IL-6 (7,239 – 16,423 pg/ml) and IL-10 (81 – 11,915 pg/ml), although two of the three also had low levels of IL-1" (24 pg/ml, 42 pg/ml).

Eighteen children were diagnosed with eczema at a mean age of 4.2 years (95% CI = 3.2 – 5.2). Children with eczema had a higher mean level of proviral load than children without eczema, although this difference was not statistically significant (P = 0.10) (Table 2). Children with eczema had a similar mean level of antibody titer and proportion with tax-specific antibodies as children without eczema (P = 0.28, P = 0.67).

All children who developed seborrheic dermatitis also developed eczema an average of eight months prior to their diagnosis with seborrheic dermatitis.

Persistant hyperreflexia was diagnosed in nine HTLV-I infected children at a mean age of 4.5 years (95% CI = 3.3 – 4.7). Children with persistent hyperreflexia had a similar mean level of proviral load, antibody titer and proportion with tax-specific antibodies as children without persistent hyperreflexia (P = 0.98, P = 0.70, P = 1.00, respectively). Eighteen of the 28 infected children developed lymphadenopathy at a mean age of 4.7 years (95% CI = 3.6 – 5.9). Children with lymphadenopathy had a similar mean proviral load level, antibody titer level and proportion with tax-specific antibodies as children without lymphadenopathy (P = 0.94, P = 0.46, P = 1.00, respectively).

Four of the 28 infected children had at least one diagnosis of severe anemia which occurred at a mean age of 4.3 years (95% CI = 0.8 – 7.9). Severe anemia was characterized as hypochromic, microcytic, which is consistent with iron deficiency. Children with severe anemia had a higher mean proviral load than children without this condition, although this difference was of borderline statistical significance (10.1 vs. 3.2 copies/100 PBMC, P = 0.08). However these two groups of children had similar mean antibody titer levels and similar proportions of these groups had tax-specific antibodies (P = 0.92, P = 0.55, respectively). Three of the four children with severe anemia also had a diagnosis of seborrheic dermatitis. Two of these children were diagnosed with severe anemia at three clinic visits separated by several intervening clinic visits without severe

anemia. Two of these children had only one diagnosis of severe anemia. All children diagnosed with anemia received iron supplements as treatment for this disorder.

Five children were diagnosed with abnormal lymphocytes at a frequency of $\geq$ 3.0%. Children with abnormal lymphocytes had a lower mean proviral load than children without this diagnosis (P = 0.08). However children with and without abnormal lymphocytes had similar mean levels of antibody titers and proportions with tax-specific antibodies (P = 0.79, P = 0.24, respectively).

Eight children had diagnoses of eosinophilia. Children with eosinophilia had a similar mean level of proviral load and proportion of tax-specific antibodies as children without eosinophilia (P = 0.66, P = 0.63)). Mean level of antibody titer was lower in children without eosinohilia compared to children without eosinophilia, although this difference was not statistically significant (P = 0.15).

The distribution of HLA Class II alleles among children by health outcome status is shown on Table 3. There were no associations between any health outcomes and HLA Class II alleles DRB1*1101, DQB1*0301, DQB1*0602.

Discussion

Our analysis revealed that level of HTLV-I proviral load measured approximately 12 months post-HTLV-I infection was higher among children who developed seborrheic dermatitis compared to children who did not develop this health outcome, although of borderline statistical significance. Mean antibody titer level was higher among children who developed seborrheic dermatitis, although this difference did not approach statistical

significance. All children who developed seborrheic dermatitis demonstrated antibody against tax in pre-diagnostic specimens, compared to 67% of other children. With respect to proviral load and tax-specific antibody, the children with seborrheic dermatitis were indistinguishable from the one child who developed infective dermatitis in this cohort. These data suggest a role for HTLV-I proviral load in the pathogenesis of seborrheic dermatitis.

Seborrheic dermatitis is a mild inflammatory dermatitis of generally healthy persons and is associated with increased colonization by *Pityrosporum Ovale (P. ovale)*, a lipophilic yeast that naturally occurs on the human skin [20]. It is believed to be hormonally triggered, occurring primarily in infancy due to high levels of maternal hormones, and again in puberty. In infants, seborrheic dermatitis presents as a thick, greasy scaling of the scalp known as "cradle cap". In adolescents it presents as eczema of scalp, external ears, axillae and groin and is often complicated by secondary impetigo or candidiasis [21]. Seborrheic dermatitis accounts for 11% of all incident cases of skin disease at pediatric dermatology clinics in Kingston, Jamaica [22].

More complicated seborrheic dermatitis is associated with another retrovirus, human immunodeficiency virus (HIV). Diseases of the skin are often early clinical signs of HIV infection and increase in severity with increasing immunodeficiency. Severe generalized seborrheic dermatitis has been reported in 26% to 45% of adults with AIDS-related complex (ARC) and 33% to 83% of adults with AIDS [23,24]. In a case-control study of HIV infected and uninfected women, high HIV viral load was a significant risk factor for skin abnormalities that included seborrheic dermatitis [25].

In children, HIV infection is associated with a wide variety of fungal, bacterial and viral infections of the skin. Severe seborrheic dermatitis involving the scalp, nasolabial folds, the skin behind the ears and the axillae is documented in HIV-infected children between the ages of two and five years of age. The morphology and distribution is similar to that seen in HIV-infected adults, and is considered highly unusual in childhood [26]. Although there is a dearth of data on HIV viral load and seborrheic dermatitis in children, it is possible that, like adults, HIV viral load plays a role in the unusual physical distribution of seborrheic dermatitis in children ages two to five years of age. Our data suggest that HTLV-I viral load also plays a role in the unusual physical distribution of seborrheic dermatitis among HTLV-I infected children.

The high serum levels of cytokines IL-6 or TNF-" and IL-10 in three of the five HTLV-I infected children with serum available at time of diagnosis with seborrheic dermatitis may represent an innate immune response to their infection with *P. ovale*. Cytokines TNF-" and IL-6, along with IL-1 are released by phagocytes in a three-phased response to bacterial or fungal infections. One phase, the containment and elimination of the pathogen, is regulated by IL-10 [27]. The second phase involves the synthesis of acute-phase proteins by hepatocytes, C-reactive protein (CRP) and mannon-binding lectin (MBL), to opsonize bacteria and fungi and activate complement [27]. The third phase is the induction of increased production of neutrophils. Ultimately the acute-phase is followed by intitiation of adoptive immunity [27].

Early adoptive immunity involves the differentiation of naïve CD4+ T cells into either TH-1 or TH-2 cells which determine whether the immune response is dominated by activated macrophages or antibody production, respectively. Although not clearly

understood, the predominant response is profoundly influenced by cytokines present during the initial T-cell activation phase. CD4+ T cells activated in the presence of IL-6 and IL-10 promote the development of TH-2 cells and inhibit the generation of TH-1 cells [27]. Thus, the innate immune response to P. ovale infection should predispose to a TH-2 adoptive immune response, which should effectively target P. ovale infection. However, the immune response that follows production of these cytokines is insufficient to control the fungus, evidenced by development of seborrheic dermatitis. There may be an underlying immune dysregulation resulting in failure to mount an effective TH-2 immune response that predisposes to fungal infections in HTLV-I infected children. HTLV-I is associated with a marker of cellular immune deficiency in adults. Adult HTLV-I carrier status has been associated with anergic response to purified protein derivative (PPD) [28]. Anergy has been associated with increased levels of sCD23, a marker of TH-2 immune response [29]. Anergy has not been studied in HTLV-I infected children.

The association of severe anemia with elevated proviral load in pre-diagnostic specimens is interesting. Iron deficiency anemia may be due to parasitic infection or poor nutrition. Parasitic infection in children was reported by only a few mothers in this study, however stool specimens were not examined so a valid measure of parasitic infection was not obtained. Anemia of iron deficiency was detected by Sweet and Walshe in Jamaican children with infective dermatitis diagnosed in the 1960's prior to the discovery of HTLV-I [30,31]. Children with infective dermatitis were reported to have abnormally low albumin levels compared to atopic children, supporting a role for

nutrition in severe anemia [10]. However 44% of these children also had parasites detected by stool microscopy, including *Ascaris* (roundworm), *Ancylostoma* (hookworm), and *Trichuris* (whipworm) [10]. The prevalence figures for *Ancylostoma* and *Trichuris* in school-aged children in Jamaica are 6% and 38% - 50%, respectively [32]. Although *Ancyclostoma* has a relatively low prevalence, infections of low worm burden are associated with iron deficiency anemia [33]. Conversely, heavy worm burdens of *Trichuris* infection, which has a much higher prevalence in this population, has also been associated with iron deficency anemia [34]. Either of these parasites may be associated with severe anemia in these children.

In summary, a high pre-diagnostic level of HTLV-I proviral load measured approximately 12 months post-infection was associated with development of seborrheic dermatitis at a median age of five years in a cohort of infected children followed clinically since six weeks of age. Children with seborrheic dermatitis were also diagnosed with eczema. Pre-diagnostic level of proviral load was associated with development of severe anemia in four children, three of whom also had diagnoses of seborrheic dermatitis. These three children had the highest levels of proviral load detected in this study. These data support the extension of HTLV-I associated diseases in children to include seborrheic dermatitis. Additionally, these data suggest that further study of the role of anemia in the pathogenesis of HTLV-I-associated diseases is warrented.

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Table 1. HTLV-I Viral Markers and Cytokine Levels in 28 HTLV-I Infected Children from Jamaica

Subjects	Age (months) at Infection	Proviral DNA Load	Antibody Titer	Anti-tax Antibody	IFN-γ	TNF-α	IL-1α	IL-4	IL-6	IL-10
1	9.9	4.4	62,797	+	UD	UD	UD	UD	UD	UD
2	14.1	9.7	21,675	+	UD	UD	UD	UD	UD	UD
3	6.9	3.6	6,217	-	UD	UD	0.1	UD	743	UD
4	26.7	13.3	5,293	+	UD	3027	134	UD	424	31
5	21.2	3.7	375,116	+	UD	UD	UD	UD	UD	UD
6	8.3	14.2	4,837	+	UD	UD	UD	UD	UD	UD
7	17.3	2.9	12,183	+	UD	UD	UD	UD	UD	UD
8	12.8	7.4	390,625	+	UD	UD	UD	UD	UD	UD
9	21.1	6.7	47,376	+	UD	UD	UD	UD	UD	UD
10	8.5	16.1	46,812	+	UD	UD	UD	UD	UD	UD
11	9.2	0.4	274	-	UD	UD	UD	UD	UD	UD
12	15.9	15.3	390,625	+	UD	UD	UD	UD	UD	UD
13	21.9	0.4	680	-	UD	UD	UD	UD	UD	UD
14	7.6	3.2	1,157	+	UD	UD	UD	UD	UD	UD
15	10.2	6.7	18,205	+	UD	UD	UD	UD	UD	UD
16	9.7	7.1	390,625	+	UD	2345	15	UD	20477	UD
17	3.7	1.5	266	-	UD	1088	30	UD	15507	UD
18	13.3	0.4	3,482	-	UD	UD	UD	UD	UD	UD
19	12.0	1.8	1,961	-	UD	570	46	UD	14921	13
20	14.9	4.7	6,733	+	UD	UD	UD	UD	UD	UD
21	17.6	7.6	45,688	+	21	UD	UD	UD	UD	UD
22	7.1	2.2	22,086	+	UD	UD	UD	UD	UD	UD
23	12.0	0.3	284	-	UD	UD	UD	UD	UD	UD
24	21.2	9.6	129,115	+	UD	1649	134	UD	UD	186
25	12.4	8.6	5,216	+	UD	UD	UD	UD	UD	UD
26	18.3	3.2	17,265	+	UD	UD	UD	UD	4.1	UD
27	25.2	0.5	7,647	+	26	UD	UD	UD	UD	UD
28	7.9	15.3	17,172	+	UD	UD	UD	UD	510	UD

Table 2. Distribution of Viral Markers by Health Outcomes

Health Outcomes	No. children	Mean proviral copies/ 100 PBMC	95% CI	Mean antibody titer*	95% CI	Prop. with tax antibody
Seborrheic Dermatitis						
Yes	**7**	**7.5**	**(4.3 – 13.0)**	**31.0**	**(11.1 – 86.7)**	**100.0**
No	**21**	**2.8**	**(1.6 – 4.9)**	**8.9**	**(3.3 – 24.2)**	**66.7**
		(P = 0.08)		**(P = 0.15)**		**(P = 0.14)**
Eczema						
Yes	**18**	**4.7**	**(2.8 – 8.1)**	**16.0**	**(6.2 – 41.1)**	**77.8**
No	**10**	**2.2**	**(0.9 – 4.9)**	**7.5**	**(1.6 – 33.9)**	**70.0**
		(P = 0.10)		**(P = 0.28)**		**(P = 0.67)**
Hyperreflexia						
Yes	**9**	**4.1**	**(1.5 – 9.3)**	**18.9**	**(4.1 – 87.7)**	**77.8**
No	**17**	**3.9**	**(2.2 – 7.0)**	**13.5**	**(5.2 – 34.9)**	**82.4**
		(P = 0.98)		**(P = 0.70)**		**(P = 1.00)**
Lymphadenopathy						
Yes	**8**	**3.4**	**(1.8 – 6.3)**	**9.5**	**(4.5 – 80.1)**	**72.2**
No	**10**	**4.0**	**(2.1 – 7.9)**	**19.1**	**(3.5 – 25.4)**	**80.0**
		(P = 0.94)		**(P = 0.46)**		**(P = 1.00)**
Anemia						
Yes	**4**	**10.1**	**(4.7 – 21.6)**	**18.2**	**(1.5 – 221.3)**	**100.0**
No	**22**	**3.2**	**(1.9 – 5.5)**	**14.7**	**(6.2 – 34.7)**	**77.3**
		(P = 0.08)		**(P = 0.92)**		**(P = 0.55)**
Abnormal Lymphs (≥3.0%)						
Yes	**5**	**1.5**	**(0.4 – 5.4)**	**9.1**	**(1.2 – 64.9)**	**60.0**
No	**21**	**4.8**	**(2.9 – 7.8)**	**17.2**	**(7.0 – 41.8)**	**85.7**
		(P= 0.07)		**(P = 0.79)**		**(P = 0.24)**
Eosinophilia						
Yes	**8**	**3.9**	**(1.8 – 8.5)**	**6.1**	**(1.8 – 20.3)**	**75.0**
No	**18**	**3.8**	**(2.0 – 7.1)**	**22.8**	**(8.5 – 84.2)**	**83.3**
		(P = 0.66)		**(P = 0.15)**		**(P = 0.63)**

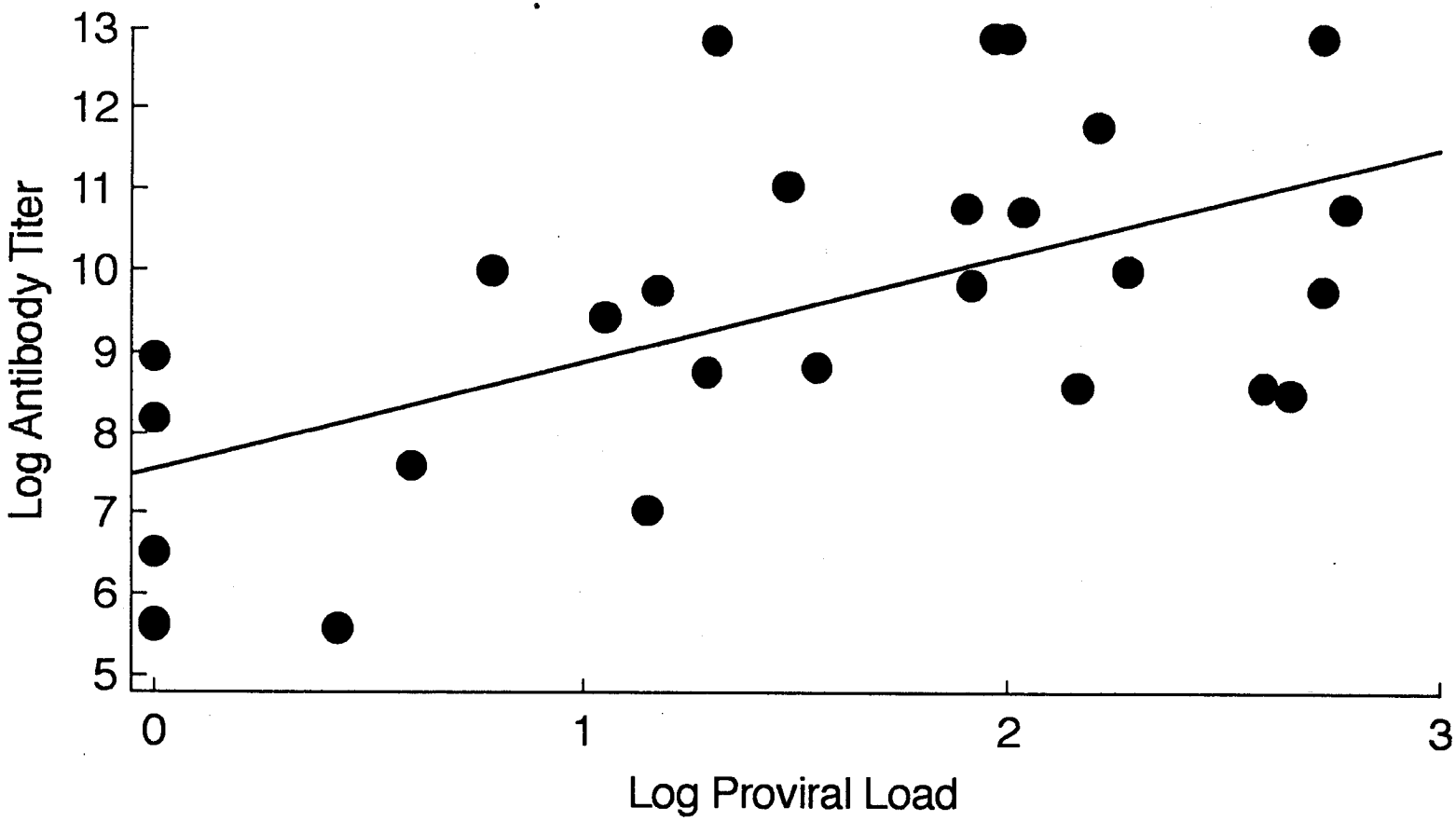
PBMC = peripheral blood mononuclear cells; Prop = proportion; *titer is in thousands

Table 3. Distribution of HLA-Class II Alleles by Health Outcome

Health Outcome	Number Children	DRB1*1101 No. with allele (%)	DQB1*0602 No. with allele (%)	DQB1*0301 No. with allele (%)
Seborrheic Dermatitis				
Yes	7	0 (----)	1 (14.3)	2 (28.6)
No	21	2 (9.5)	2 (9.5)	7 (33.3)
		(P = 1.00)	(P = 1.00)	(P = 1.00)
Eczema				
Yes	18	2 (11.1)	2 (11.1)	8 (44.4)
No	10	1 (10.0)	1 (10.0)	2 (20.0)
		(P = 1.00)	(P = 1.00)	(P = 0.25)
Lymphadenopathy				
Yes	7	0 (----)	0 (----)	1 (14.3)
No	21	3 (14.1)	3 (14.3)	9 (42.9)
		(P = 0.55)	(P = 0.55)	(P = 0.36)
Anemia				
Yes	4	0 (----)	9 (----)	1 (25.0)
No	22	2 (9.1)	3 (13.6)	8 (36.4)
		(P = 1.00)	(P = 1.00)	(P = 1.00)
Abnormal Lymphocytes (≥ 3%)				
Yes	5	0 (----)	0 (----)	2 (40.0)
No	21	2 (9.5)	3 (14.3)	7 (33.3)
		(P = 1.00)	(P = 1.00)	(P = 1.00)
Eosinophilia				
Yes	8	1 (12.5)	2 (25.0)	2 (25.0)
No	18	1 (5.6)	1 (5.6)	7 (38.9)
		(P = 0.53)	(P = 0.22)	(P = 0.67)

no. = number

Correlation of HTLV-I Antibody Titer and Proviral Load



r = 0.57 p = 0.002

Overall Discussion

Morbidity associated with human T-lymphotropic virus type-I (HTLV-I) among adults includes a rare T-cell malignancy called adult T-cell leukemia/lymphoma (ATL) and a chronic, degenerative neurologic disease called HTLV-I associated myelopathy or tropical spastic paraparesis (HAM/TSP) [1,2]. More recently HTLV-I was associated with an inflammatory eye disease, uveitis [3]. Additional diseases and conditions suggested to be associated with HTLV-I include arthropathy, Sjögren's syndrome, lymphadenitis and polymyositis [4-7]. Other conditions associated with HTLV-I infection among adults include asthma, cardiac abnormality, bacterial infections, anemia, lymphocytotosis and a decreased prevalence of eosinophilia [8-12]. A history of kidney disease, detection of lymphadenopathy and gait abnormality at physical examination were also associated with HTLV-I infection among adults [10].

HTLV-I associated morbidity in childhood has not been well studied. Children under the age of ten years have the lowest prevalence of any age group in endemic areas. Although < 1.7% of this age group is seropositive in Jamaica, the clinical significance of childhood infection is based on its role in adult HTLV-I associated diseases [13]. A case-control study of non-Hodgkin's lymphoma (NHL) in Jamaica showed that 70% of the incidence of T-cell NHL in persons under the age of 40 years could be explained by HTLV-I infection [14]. Given the long latency of this disease, these data suggested that infection early in life was associated with developing ATL. A subsequent family study of ATL patients supported this contention by showing that greater than 90% of mothers of ATL patients were HTLV-I seropositive [15]. Infective dermatitis is associated with HTLV-I infection in early childhood [16]. Case reports of five adults with either ATL or HAM/TSP with documented childhood histories of infective dermatitis suggest that

infective dermatitis may be a marker of risk for development of both ATL and HAM/TSP [17-19]. However, there may be other health effects associated with HTLV-I infection in children. A primary objective of the present study was to identify the morbidity associated with childhood HTLV-I infection. A secondary objective among HTLV-I infected children was to determine if pre-diagnostic viral and immunologic markers, as well as genetic markers played important roles in disease development.

Analysis of data addressing the two objectives of this study was based on a cohort of 28 HTLV-I infected and 280 uninfected children born to 308 mothers who enrolled in a study of maternal-child transmission of HTLV-I [20]. All mothers were attending one of two antenatal clinics in Kingston, Jamaica at the time of their enrollment between January, 1989 and September, 1990. The 308 children received physical examinations and phlebotomy at clinic visits scheduled every six weeks for the first three months of life, every three months until age two years and every six months thereafter until ten years of age.

To address the primary objective, several health outcomes were hypothesized to be associated with HTLV-I infection in children. Specifically, HTLV-I infected children were hypothesized to be at increased risk of developing eczema and seborrheic dermatitis, as these represent differential diagnostic alternatives of infective dermatitis. Additionally, HTLV-I infected children were hypothesized to be at increased risk of developing lymphadenopathy and lymphocytosis, features of ATL which have been associated with HTLV-I seropositive status in adults [10,11], and abnormal lymphocytes, another feature of ATL. Infected children were examined for increased risk of gait abnormality, which was reported to be associated with HTLV-I in adults [10], as well as

other features of HAM/TSP, including hyperreflexia and increased muscle tone. Other abnormalities hypothesized to be associated with HTLV-I in children included anemia, asthma, bacterial infections, cardiac abnormality and low percent eosinophils, which were associated with HTLV-I among adults [8-12]. Based on clinical observations that children with HTLV-I associated infective dermatitis were of small size for their age, HTLV-I infected children were hypothesized to be at increased risk of low body mass index (BMI). Cox proportional hazards regression was used to compare incidence rates for these health outcomes between the 28 HTLV-I infected and 280 uninfected children, with adjustment for sex and maternal income when indicated.

The results of our analyses suggested that HTLV-I infected children have significantly increased incidence rates of seborrheic dermatitis, eczema, and persistent hyperreflexia compared to uninfected children. Additionally, infected children have non-significantly increased incidence rates of lymphadenopathy, severe anemia, abnormal lymphocytes and a non-significantly decreased rate of eosinophilia compared to uninfected children. With the exception of the skin diseases, all of these HTLV-I associated health effects have previously been reported in adults suggesting that subclinical effects of HTLV-I infection are similar in children and adults.

Based on these results, the secondary objective was to examine the 28 HTLV-I infected children to assess whether viral, immunologic and host genetic markers were associated with development of the health effects associated with HTLV-I infection in childhood. Viral markers included HTLV-I proviral load, antibody titer and tax-specific antibody. Targeted health outcomes were hypothesized to be associated with high levels of proviral load and antibody titer based on reported associations of these markers with

ATL and HAM/TSP. A high proportion of tax-specific antibody was hypothesized to be associated with development of HTLV-I associated diseases and conditions based on the correlation of tax antibody with both proviral load and antibody titer in adults.

Immunologic markers refer to serum cytokines that represent T-helper type 1 (TH-1) and T-helper type 2 (TH-2) immune responses. TH-1 cytokines included IFN-γ, TNF-α, IL-1α and IL-6; TH-2 cytokines included IL-4 and IL-10. HTLV-I infected children who developed one of the diseases or conditions under study were hypothesized to have elevated TH-2 cytokines in prediagnostic specimens. Host genetic markers included three human leukocyte antigen (HLA) Class II alleles that have previously been associated with ATL or HAM/TSP (DRB1*1101; DQB1*0301; DQB1*0602).

Results of the secondary analysis suggested that increased incidence rates of seborrheic dermatitis and severe anemia were associated with elevated HTLV-I proviral load in pre-diagnostic specimens, although these associations were of borderline statistical significance. Pre-diagnostic cytokine levels were not associated with proviral load or any targeted diseases or conditions, with the exception of the one child who developed infective dermatitis and seborrheic dermatitis. However three of five children with seborrheic dermatitis who had available specimens for testing had elevated levels of TNF-" or IL-6 and IL-10 at the time of diagnosis. None of the HLA Class II alleles were associated with diseases or conditions in this study.

The prospective design of this study afforded the opportunity not only to examine the temporal relationships between viral markers and health outcomes, but also the timing of the occurrence of these health outcomes. All children with seborrheic dermatitis developed lymphadenopathy an average of three months prior to diagnosis of seborrheic

dermatitis. Three of the seven children who developed seborrheic dermatitis also developed severe anemia. Severe anemia preceded seborrheic dermatitis by an average of seven months. Four of the seven children with seborrheic dermatitis also had persistent hyperreflexia of the lower limbs diagnosed an average of six months before diagnosis of seborrheic dermatitis. This longitudinal perspective suggests considerable overlap of abnormalities among children with seborrheic dermatitis. Evidence of relationships between these abnormalities was seen in the one infected child in this cohort who developed infective dermatitis. That child had been diagnosed with severe anemia and hyperreflexia prior to his diagnosis with infective dermatitis, and lymphadenopathy and abnormal lymphocytes subsequent to his diagnosis with infective dermatitis [21]. HTLV-I associated skin disease may be the most obvious marker of a range of clinical abnormalities potentially linked with subsequent hematological and neurological sequelae. The recent report of ten cases of HAM/TSP diagnosed over a ten year period in Brazilian children under the age of 18, described histories of infective dermatitis in seven of the children [22]. A report of ten pediatric cases of ATL in Brazil described the majority as having skin disease at presentation [23], and nine cases of HTLV-I associated ATL in children from varying geographic areas are further evidence of the range of HTLV-associated clinical abnormalities that may be seen in children [24-29].

The ages at which seborrheic dermatitis was diagnosed in this study differed from those at which seborrheic dermatitis is usually diagnosed in infancy or adolescence [30]. The mean age of diagnosis in our study was five years (95% CI = 3.9 – 6.0 years). It is plausible that HTLV-I associated seborrheic dermatitis, like HIV-associated seborrheic dermatitis, has an unusual age at presentation [31,32]. This parallel between HTLV-I-

and HIV-associated seborrheic dermatitis suggests a role for underlying immune suppression.

A secondary objective of our study was to examine relationships between elevations of serum cytokine levels and clinical abnormalities associated with HTLV-I infection. We did not find evidence that elevation of serum cytokine levels 12 months post-infection correlated with proviral load. Additionally, pre-diagnostic cytokine levels did not appear to be generally associated with subsequent development of any of the targeted health effects associated with HTLV-I in this study. However, the single child who developed infective dermatitis had the highest pre-diagnostic level of TNF-α as well as elevated levels of IL-1α, IL-6 and IL-10. Of the five infected children with available serum at time of diagnosis with seborrheic dermatitis, three children had elevated levels of IL-10, and either TNF-α or IL-6. No IFN-γ or IL-4 was detected among these cases. Although these results are based on a small number of children, elevation in levels of these cytokines may represent an innate immune response to infection with *Pityrosporum Ovale (P. ovale)*, the yeast pathogen associated with seborrheic dermatitis. Elevated TNF-α and IL-6 may be produced by skin keratinocytes in response to colonization of yeast on the skin surface, as suggested by in-vitro models [33]. Thus an elevated inflammatory response to overgrowth of yeast (or *Staph Aureus or β-hemolytic strep infection* in the case of infective dermatitis) could result in the symptoms which characterize these skin diseases. This possibility is supported in the case of infective dermatitis by the rapid effect of antibiotics in ameliorating symptoms of this disease.

Several outstanding questions remain regarding the pathogenesis of HTLV-I associated diseases in children. Our study supports an association of HTLV-I infection

with seborrheic dermatitis. However the unusual age of onset of seborrheic dermatitis in our study raises two possibilities. HTLV-I associated seborrheic dermatitis, like HIV-associated seborrheic dermatitis, occurs at different ages than conventional seborrheic dermatitis; alternatively HTLV-I associated seborrheic dermatitis is a mild form of HTLV-I associated infective dermatitis which occurs between two and 16 years of age [34, LaGrenade L, personal communication]. To address this question, a case-control study is planned that will enroll all incident pediatric patients with diagnoses of eczema, seborrheic dermatitis and infective dermatitis (cases) $\leq$ ten years of age and healthy controls matched at a 3:1 ratio by age, sex, and socioeconomic status. Cases will be ascertained from three pediatric dermatology clinics in Kingston, Jamaica and controls will be ascertained from the same comprehensive health clinics from which the cases are referred. The cases will be composed of a series of approximately 738 eczema cases that include 198 children with seborrheic dermatitis, 135 children with infective dermatitis and 405 children with 'other' eczema diagnoses. Cases within separate diagnostic categories will be compared to controls with respect to HTLV-I prevalence, and odds ratios (OR) will be computed describing these associations. Clinical definitions of HTLV-I associated eczema and seborrheic dermatitis will be obtained by comparing several demographic, biologic and immunologic parameters between HTLV-I infected and uninfected cases within diagnostic categories. Demographic factors will include age, sex and socioeconomic status. Biologic and immunologic parameters will include complete blood count and differential, immunoglobulin levels, T-cell subsets, activated T-cells, cytokines, serum albumen and other nutritional markers, infections with parasitic, bacterial and fungal pathogens.

A second question raised by our data is whether the high level of HTLV-I proviral load 12 months post-infection in children with seborrheic dermatitis and eczema decreases, remains at the same level, or increases throughout their clinical observation period. In adults who became infected following transfusion with HTLV-I seropositive blood units, their proviral load and antibody titer essentially reached a plateau 12 months post-infection, maintaining that level over the course of their observation period [35]. Proviral load and antibody titer in the one child from this cohort who developed infective dermatitis at age 46 months increased in linear fashion to the end of his observation at 78 months [21]. This one case suggests that disease results from an inability to achieve immunologic control of viral reproduction, which may be an important determinant of pathogenesis. Viral markers will be assessed in longitudinal specimens of all HTLV-I infected children to determine if proviral load and antibody titer continue to increase over time in those with seborrheic dermatitis and eczema compared to HTLV-I carrier children.

A related question concerns the immunologic events that are occurring between the initial HTLV-I proviral load 12 months post-infection and the subsequent elevation of serum pro-inflammatory cytokine levels at time of diagnosis. Understanding these events could inform us of the pathogenic mechanism involved in seborrheic dermatitis. Examination of T-cell subsets in the child with infective dermatitis showed that the frequency of activated T-cells was elevated prior to development of disease, as was his ratio of CD4+ T-cells to CD8+ T-cells, both of which subsequently declined after disease development, probably due to treatment with corticosteroids and antibiotics [21].

However levels of both parameters rebounded subsequent to treatment, perhaps due to periods when the child went off treatment.

We suggest the hypothesis that a high initial level of proviral load may have an adverse effect on the host immune response rendering it unable to control viral replication. The increased number of CD4+ T-cells may represent a proliferation of HTLV-I infected cells, or, as supported by the number of CD4+CD25+ T cells, an increased number of activated T cells which are the target of the HTLV-I virus. It is possible that a high level of viral replication or gene expression overwhelms the immune system in children, allowing for the colonization of other pathogens (i.e. yeast in the case of seborrheic dermatitis, or *S. aureus/β-hemolytic strep* in the case if infective dermatitis).

In order to determine if an initially high level of viral replication is overwhelming the immune response, additional laboratory testing will be conducted using new technology to measure the thymic production of naïve T-cells, as well as memory T-cells. A recent study comparing HTLV-I uninfected and infected asymptomatic carriers found that carriers under age 50 years had a significantly lower percentage of naïve T-cells but a higher frequency of memory cells compared to age-matched uninfected persons [36]. The number of memory cells was highly correlated with proviral load, which was thought to be due their proliferation in response to chronic HTLV-I infection. Although the frequency of naïve T-cells was not correlated with proviral load in this study, in the setting of HIV, the reduction of proviral load by HAART therapy resulted in an increase in the numbers of naïve T-cells. Lower percentage of naïve T cells in HIV infection was thought to reflect the impaired function of the thymus. This contention was supported by

data obtained from measuring T cell receptor rearrangement excision cycles (TRECs), which are generated during early T lymphopoiesis. TREC levels were significantly lower in HTLV-I carriers than uninfected persons suggesting that carriers produce a low proportion of naïve T cells which could be an indicator of thymic atrophy [36].

It is possible that an initially high proviral load is associated with an increased proportion of memory T-cells but a decreased proportion of naïve T-cells. A decreased proportion of naïve T-cells may impair the immune response to yeast and bacterial infections, resulting in their colonization. Colonization by these pathogens may result in a related increase in activated T-cells, providing target cells for HTLV-I infection leading to a subsequent increase in HTLV-I proviral load over time. The colonization of yeast has been demonstrated to induce the secretion of specific inflammatory cytokines *in vitro*, which in mouse models have been demonstrated to impede production of naïve T-cells [36]. Increases in inflammatory cytokines in children at the time of diagnosis with seborrheic dermatitis could be associated with further decreased production of naïve T-cells, setting the stage for risk of developing ATL or HAM/TSP.

To conclude, HTLV-I infection in childhood may be associated with seborrheic dermatitis and eczema, increasing the range of childhood skin diseases associated with HTLV-I. Alternatively, seborrheic dermatitis may be a mild form of infective dermatitis. Further study is required to confirm these associations and clinically define these HTLV-I associated skin diseases. HTLV-I infection was also significantly associated with persistent hyperreflexia, and non-significantly associated with increased incidence rates of lymphadenopathy, severe anemia and abnormal lymphocytes, findings which

were previously associated with HTLV-I among adults. These findings suggest that subclinical abnormalities are similar in children and adults infected with HTLV-I.

Seborrheic dermatitis was associated with elevated HTLV-I proviral load detected 12 months post-infection suggesting that proviral load may be an important determinant of disease development. Severe anemia consistent with iron deficiency was also associated with elevated proviral load. Additionally, children with seborrheic dermatitis had at least one other condition including lymphadenopathy, abnormal lymphocytes, persistent hyperreflexia or severe anemia. The overlap of seborrheic dermatitis with these conditions suggests that subclinical abnormalities may contribute to development of seborrheic dermatitis. Seborrheic dermatitis was associated with high levels of pro-inflammatory cytokines at the time of diagnosis, which may represent an innate immune system response to yeast infection. However, these specific inflammatory cytokines may result in a dysregulated immune response to HTLV-I infection. Further study is needed to determine the effect of high initial levels of proviral load on the immune systems response to infection in children with HTLV-I associated skin diseases.

Public Health Implications

Our study suggests that HTLV-I infection in early childhood is associated with pediatric seborrheic dermatitis. Additionally, children with seborrheic dermatitis appear to have higher levels of HTLV-I proviral load. Although we cannot predict the health consequences for these children, we can speculate about potential consequences based on what is known about another pediatric skin disease. Infective dermatitis is associated with childhood infection with HTLV-I and occurs at approximately the same age as seborrheic dermatitis. ATL is believed to be associated with childhood HTLV-I

infection. Infective dermatitis has been suggested to be a marker of increased risk for development of ATL (and HAM/TSP) in adulthood based on case reports. Further studies are needed to confirm the association of HTLV-I with seborrheic dermatitis, and to determine whether proviral load continues to increase over time in children with this diagnosis. Such documentation could increase the plausibility that pediatric seborrheic dermatitis may predispose to adult HTLV-I associated diseases characterized as having high levels of HTLV-I proviral load. However, there are public health measures that can be debated now in advance of confirmation. These measures include both primary and secondary prevention.

Primary prevention measures would have as their goal the prevention or diminishing of HTLV-I infection in children. It is known that childhood infection results from maternal-child transmission predominantly via breast-feeding. In a study of risk factors for HTLV-I infection in children, 32% of children who breastfed for $\geq$ 12 months became infected compared to 9% of children who breastfed for < 12 months [20]. Thus any intervention to prevent childhood infection would involve recommendations to either limit breast-feeding duration or replace breast-feeding with bottle-feeding.

In Japan, where HTLV-I is also endemic, a national policy has successfully been implemented to recommend bottle-feeding to HTLV-I infected women who give birth. This intervention has resulted in a decrease in HTLV-I prevalence in children. However, Jamaica is a developing country and as such must be concerned about the potential negative consequences of diminishing or replacing breast-feeding. Importantly, breast-feeding is known to protect children against diarrheal diseases and malnutrition. Additionally, breast-feeding is an inexpensive alternative to formula, the cost of which is

prohibitive for Jamaican women of low socioeconomic status. Further, such an approach would require all pregnant women to be screened for HTLV-I antibodies. This is an expensive initiative to undertake in a country that has limited resources and competing public health concerns that utilize those resources.

In an intervention study conducted in Kingston Jamaica by the University of the West Indies, in collaboration with Kagoshima University in Japan, a protocol to either continue beast-feeding or initiate bottle-feeding following an initial six months of breast-feeding was implemented. There was no significant difference in the rate of maternal-child transmission between the two treatment arms, due to continued night-time breast-feeding among mothers who had elected to bottle-feed their children. This study underscores the barriers to breast-feeding intervention as a primary means of preventing HTLV-I infection in Jamaican children.

A second approach to diminish risk of infection in children is to decrease maternal HTLV-I proviral load. High levels of maternal proviral load were independently associated with maternal-child transmission in Jamaica [37]. Diminishing maternal proviral load prior to delivery and during breast-feeding may decrease the rate of transmission of HTLV-I to offspring. This intervention has several barriers that would have to be overcome in order to be feasible. First, the effectiveness of antiretroviral agents against HTLV-I has not been firmly established. In vitro laboratory data suggest that with the exception of lamivudine (3TC), nucleoside reverse-transcriptase inhibitors (NRTIs) that included zidovodine (Zdv), didanosine (ddI), stavudine (d4T) and zalcitabine (ddC) significantly decrease HTLV-I reverse transcriptase in HTLV-I isolates [38]. Additionally, in one adult with HAM/TSP a 100-fold decrease in HTLV-I provirus

was sustained for 16 months of follow-up when treated with 3TC and Zdv [39]. Thus treatment with NRTIs is a potential intervention to explore in reducing maternal HTLV-I viral load during breast-feeding and thereby reducing risk of transmission to offspring. However, adverse events in children due to their mother's treatment during breast-feeding must be investigated beforehand.

In the event that primary prevention is not feasible, secondary prevention measures could be considered. This would involve the prevention of ATL and HAM/TSP in HTLV-I infected children at risk of disease. In order to justify such prevention efforts it is necessary to provide evidence of a causal link between markers of increased risk for such diseases and the diseases themselves. Case reports have suggested that HTLV-I associated infective dermatitis in childhood is a marker for ATL and possibly HAM/TSP [17-19]. Our data further suggest that HTLV-I associated seborrheic dermatitis may be an additional marker of these adult diseases because it is associated with early childhood infection with HTLV-I and a high level or proviral load. The longitudinal proviral load levels in the single child in our cohort who developed infective dermatitis increased in a linear fashion throughout his follow-up. Plans to test the proviral load in longitudinal samples obtained from children with HTLV-I associated seborrheic dermatitis have been initiated. If seborrheic dermatitis is associated with increasing levels of proviral load this may be a sufficient indicator of risk for more serious HTLV-I associated outcomes, as ATL and HAM/TSP patients are known to have very high levels of proviral load. Treatment with antiretroviral therapy may be the appropriate intervention to reduce proviral load and prevent potential development of ATL or HAM/TSP. However, more information is needed on the effectiveness of these therapies before administering them to

children. Additionally, the cost of such therapies over an extended period of time may be prohibitive and impractical without more convincing evidence of a causal link between these pediatric skin diseases and development of ATL or HAM/TSP. In the interim period before research provides those answers, the surveillance of children with these diagnoses is warrented. Two children with infective dermatitis have developed renal failure possibly as a result of infection with *Beta-hemolytic stretococcus* bacteria, or the accumulation of immune complexes [40]. Monitoring children for early signs of renal abnormalities will enable the implementation of early treatment. Monitoring children for signs of early ATL or HAM/TSP will allow for the accumulation of needed data establishing a causal link between markers and adult disease development. Surveillance will also provide for the easy identification of children who may benefit from antiretroviral therapy when it is deemed a safe and effective intervention in children.

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